'FLAVOR NUCLEOTIDES'

by

A. JOSEPH

A dissertation submitted in partial fulfilment for the award of MASTER OF SCIENCE Degree in FOOD TECHNOLOGY of the University of Mysore

FAO INTERNATIONAL FOOD TECHNOLOGY TRAINING CENTRE,
CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE, MYSORE-2A, INDIA

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(A.JOSEPH)

C O N T E N T S

In this Dissertation, the following abbreviations have been used :-

MSG : Mono-sodium L-Glutamate.

RNA : Ribonucleic acid

RNASE: Ribonuclease

AMP : Adenylic acid

GMP : Guanylic acid

UMP : Uridylic acid

CMP : Cytioylic acid

IMP : Inosinic acid

XMP : Xanthosine-5'-phosphate

PRPP: 5-Phosphoribosyl pyrophosphate

PP:Pyro-phosphate

RSP:Ribose-5'-Phosphate

Pi : Phosphoric acid (Inorganic Phosphate)

CHAPTER I

INTRODUCTION

"Apart from the simple stimulation of the senses, very little is known of the true function of aromatic and flavoring substances, yet their importance in the human diet is very great".- Prof.Hans Glatzel.

Importance of Flavor Potentiators

The control of flavor is of vital and continuous concern to the food processor. The development of a new product often presents a bewildering maze of flavor problems. Even after a desired flavor has been obtained, reproduction of this flavor over a period of time may become a major problem.

Variations in the quality or availability of ingredients may result in unwanted flavour changes. In a similar fashion, the introduction of a new processing technique often results in products of inferior flavor.

To achieve and maintain the preferred flavor characteristics, the food processor has had at his disposal a large number of natural essential oils and flavors plus many synthetic counterparts that could be selected and mixed to stimulate the flavor of natural food products or combination of such products.

Relatively, few pure compounds have been available, however, that could be used to blend or modify flavors. Also referred to as seasoners or flavor enhancers or flavor potentiators, such compounds contribute more to the flavor of a product by their ability to blend or modify other flavor characteristics than by the addition

of a new flavor notes. As a result, they tend to be useful in a greater variety of foods. Mono-sodium glutamate is an excellent example of a compound that functions in this manner. Its utility is widely recognized and applied by many food industries.

In recent years, it has become apparent that several naturally occurring 5'-nucleotides are also capable of modifying flavor. Because of their unique seasoning, properties and their high activity, they offer the food industry new opportunities for flavor modification and control.

The Oxford Dictionary gives as synonyms for enhance, the words, heighten, intensify, exaggerate, raise, increase and augment. Though the words 'potentiator' and 'enhancer' are well-known to pharmocologists, flavor potentiation is a new term in the vocabulary of flavor research. Flavor refers to the action of a compound, which in small quantities has, by itself, no sensory efforts but exaggerates the effect of other agents on that system (Sjostrom, 1965).

In the context of flavor, the term 'potentiator' is only a few years old. The isolation and identification of flavor potentiators is a twentieth century accomplishment, an era of research is still in its infancy. And yet, flavor potentiation is as old as cooking.

For centuries, cooks have added ingredients to foods, or prepared food in particular ways to improve flavor. Flavor secrets were handed down from generation to generation, delicate dishes were prepared without any understanding of the phenomena involved. It is only recently that we have sought to learn the reasons for these phenomena and we have sought to develop a com-

prehensive understanding of the elements of flavor and of flavor perception. This research has led to intensive work in the area of flavor potentiation and has opened doors which were not even known to exist only a few years ago.

To understand potentiation, it is necessary to cover the subject from several different vantage points. One of these is the concept of flavor itself. Flavor is a very complex concept, as (many cook and) anyone involved in this study, processing, preparation or presentation of food well knows.

Flavor is the combination of taste, feeling, and odour on receptors in the mouth and the nose. It is not a single impression, but a series of impressions, each of which may be distinct or overlaping and may last a matter of microseconds, or as in the case of some after-tastes, for many minutes.

Thus, far we have described flavor as it is perceived because flavor potentiators affect the ways, in which flavor is perceived. At the same time, however, flavor potentiators are elements of the stimuli that affect this perception. Therefore, an understanding of the flavor systems inherent in the materials we eat is also essential to an understanding of potentiators.

Their appear to be many different potentiators which may be found in foods that we eat today. Many of these have yet to be identified and isolated. At this point, only a few are being produced commercially. Important types are :

1)Salt

2)Amino Acids

3)Maltol

4)Enzymatic Enhancers

5'-Nucleotides

They are the first of the true flavor potentiators. There are currently two commercial forms of the 5'-Nuclotides - disodium 5'-inosinate and di-sodium 5'-granylate. Both are many times more potent than MSG, although it is difficult to quantify their relative potency in absolute terms, because the effects produced by the nucleotides are not quite the same as those which result from those of MSG. It has been estimated, however, that the inosinate has roughly 10-20 times the flavor enhancing ability of MSG. When MSG is effective in enhancing the flavor of foods in parts per thousand, 5'-Nucleotides are effective in concentrations of parts per billion or even less. Nucleotides act well with most of the foods that go well with MSG. In addition to requiring lower concentrations, however, the nucleotides also add effects which are not found with MSG. The acceptance of the 5'-Nucleotides by commercial users has been relatively s wift. Today, there are mixtures of two nucleotides, mixtures of this product and MSG are commercially available.

In liquid foods, they create a sense of increased viscosity. Soups have body, more 'mouthfeel'. The nucleotides season, they enhance and they are physiologically active.

There is a specific synergistic action in flavor between 5'-nucleotides and amino acids mainly MSG. This synergistic action furnishes the most important key to the development of the flavor potentiator.

CHAPTER II

HISTORY

The introduction of flavor potentiators arises from studies on Japanese foods. Since the early part of the present century, many Japanese food scientists have undertaken to isolate the flavor components from the natural foods peculiar to Japan, such as sea tangle, a kind of sea-weed and dried bonito, a kind of fish. Both sea tangle and dried bonito have been used generally for seasoning, Japanese meals, because they can be well preserved for a long time and the extracts have characteristic flavor activities. It seems natural therefore that Japanese workers had special interests in these materials.

At first Ikeda (1908,1909) chemically fractionated sea tangle extract, and isolated a crystalline flavor compound which was easily identified as MSG. As it was already known that L-glutamate was present in protein hydrolysates, economical production of MSG from plant proteins was not so difficult. In fact, immediately after Ikeda's discovery, MSG was produced commercially as the first seasoning. At present, MSG is produced not only by protein hydrolysis but also by direct fermentation or chemical synthesis and the flavoring action of MSG has also been studied extensively. MSG is now accepted as an excellent flavor enhancer or potentiator by food technologists, food processors and housewives and is used in huge volumes throughout the world.

On the otherhand, Kodama (1913) reported that the principal flavor component of dried bonito was the histidine salt of IMP. Unfortunately, this compound could not be produced economically and,

therefore, was not used commercially because both the biochemical background and the flavor property of IMP are more complicated than those of MSG. The use of IMP as a flavor potentiator was realised about fifty years later, when a relationship between chemical structure and flavor activity of nucleotides was found (Kuninaka, 1960) and a process developed for preparing these compounds from ribonucleic acid (Kuninaka et al, 1959, 1961).

Why was the commercial use of 5'-nucleotides delayed so long ? One of the reasons was the existence of three isomers of inosinic acid. There are two types of glutamate-L and D-isomers - whereas, there are 3 types of IMP - 2',-3' and 5'-isomers. L-glutamate is a natural component of protein but D-glutamate is not. Flavoring action is recognized only in L-glutamate and not in D-glutamate. The relationship between isomerism and flavoring action of IMP had never been reported, nor had experiments been made, that allow one to define the interaction between IMP and histidine. Furthermore, the biochemistry of nucleic acid is a more modern field than that of protein or carbohydrate, so the biochemical position of IMP was clarified much later than that of L-glutamic acid.

It was not clear which isomer had flavor enhancing properties. To solve the problem, Kuninaka et al (1964) prepared these isomers and checked their flavor effects. Inosine 2'-monophosphate (2'-IMP) and inosine 3'-mono-phosphate (3'-IMP) were prepared from ribonucleic acid and 5'-IMP was isolated from animal muscle tissue. The conclusion was that among the three isomers,only 5'-IMP had flavor activity and histidine was not necessary. Hypoxantine, inosine and ribose 5-phosphate had no

flavor activity. So, both ribosidic and 5' phosphomonoester linkages are essential for flavoring action. Furthermore, in the structure of 5'-IMP, the hydroxyl group at the 6-position was confirmed to be essential for flavor activity. If the hydroxyl group is replaced by an amino group, flavor activity decreases sharply. On the otherhand, hydrogen at the 2-position could be replaced by another group such as hydroxyl or amino group, without much change in flavor activity. In other words, not only 5'-IMP, but also 5'-GMP and 5'-XMP were found to have flavor activity.

The general structure of the nucleotides which have flavor activity is given in figure:

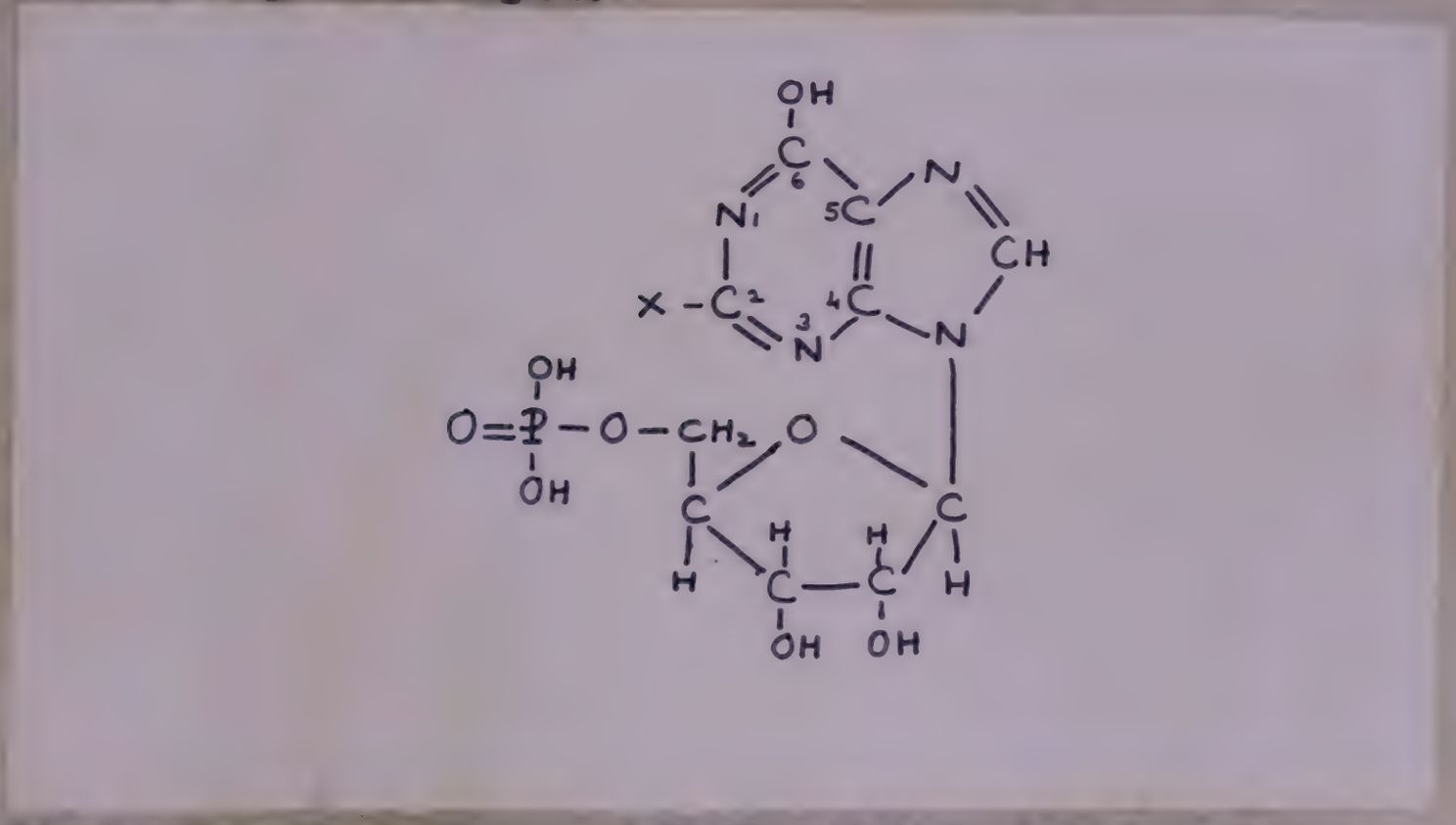
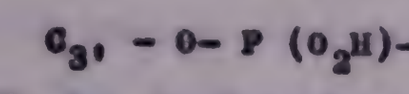


Fig.1 : General structures of the Nucleotides which have flavor activity.

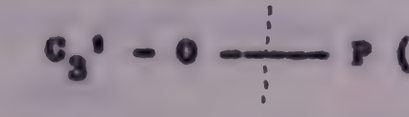
When 'X' is substituted with simple hydrogen, the resulting compound is 5'-IMP substitution of an amino group or a hydroxyl group at this position creates 5'-GMP or 5'-XMP."

Kuninaka et al, therefore, undertook to prepare 5'-IMP from RNA. Recently, it has become evident that the RNA is a

polynucleotide in which individual nucleoside residues are joined, one to the other, by phosphodiester linkages between 3'- and 5'-positions. When RNA is degraded, cleavage must be considered to occur at either the 3'-phosphodiester linkages



or the 5'-phosphodiester linkages.



Most of the RNA-degrading enzymes split 3'-phosphodiester linkages in RNA, giving rise to nucleoside - 2', 3'-cyclic phosphates or 3'-nucleosides. Alkali splits the same linkages, giving rise to nucleoside -2', 3'-cyclic phosphates which are further cleaved to form 2' and 3' nucleotides.

Only, the so called non-specific phosphodiesterases of snake venom and intestinal mucosa had been demonstrated to split 5'-phosphodiester linkages in RNA, giving rise to 5'-nucleotides. However, it is very difficult to obtain large amounts of these enzymes free of phosphemone esterase activity. Thus, chemical or enzymic production of 5'-nucleotides from RNA was very difficult and economically feasible production thereof was quite impossible.

Kuninaka et al (1957) demonstrated that 5'-phosphodiesterases which split 5'-phosphodiester linkages in RNA, giving rise to 5'-nucleotides were formed by several microorganisms such as penicillium citrinum. Using the microbial 5'-phosphodiesterase, which can be obtained in large amounts, 5'-AMP, 5'-GMP, 5'-IMP could be easily obtained in large amounts. 5'-IMP, 5'-GMP, 5'-CMP, 5'-UMP be produced industrially from yeast RNA. 5'-IMP could be easily obtained

by de-aminating 5'-AMP. Omura et al (1958) also demonstrated similar enzymes independently in several microorganisms such as streptomyces, Thus 5'-IMP has been produced microbiologically and used as a new seasoning. Furthermore, establishment of this, RNA degradation process also led to recognition of the flavor of 5'-GMP, one of the RNA degradation product for the first time. 5'-GMP was found to have stronger flavor than 5'-IMP (Sakaguchi et al 1958, Kuninaka 1960).

Kuninaka (1956, 1957, 1959) demonstrated that Aspergillus ribosidase specifically attacks the flavor nucleotides 5'-IMP, 5'-GMP and 5'-XMP, as well as their nucleotides. It may be suggested that there is an anology between sensory specificity and enzyme specificity. In fact, the study of specificity of ribosidase enzyme action on the nucleotides also led to the discovery of the specific flavor activities of 5'-GMP and 5'-XMP. According to Hosoi (1961), mosquitoes like neither MSG nor the flavor nucleotides, 5'-IMP and 5'-GMP, but specifically like 5'-AMP and its derivatives. It is interesting that 5'-AMP is not attacked by Aspergillus ribosidase which attacks the flavor nucleotides, but is specifically attacked by Azotobacter ribosidase (Huwitz et al, 1957). Thus, 5'-IMP and 5'-GMP are preferred by Aspergillus enzyme as well as human while 5'-AMP is preferred by Azotobacter enzyme as well as mosquito.

Among the three flavor nucoleotides, 5'-XMP is not produced commercially, because its flavor activitiy is weaker than that of 5'-GMP or 5'-IMP.

MSG and 5'-IMP were first reported as flavor components of natural foods, and their then introduced as flavor potentiators. Actually, various foods contain them. On the otherhand, the flavor activity of 5'-GMP was first assumed from the similarity in its chemical structure to 5'-IMP and it was then actually proved when it was produced from ribonucleic acid as a by product of 5'-IMP (Kuninaka, 1960). Therefore, 5'-GMP may be thought to have been introduced as a non-natural flavor potentiator. In fact, 5'-GMP is found to be present only in a few natural foods, such as a kind of Japanese mushroom, 'Cortinellus shittake', which contains about 100 mg. of 5'-GMP per 100 gm. The 5'-GMP content of meats is usually only about 2 mg. per 100 gm. while the 5'-IMP content is about 100 mg per 100 gm. (H.Nakajima et al, 1961).

The discovery of 5'-nucleotides as flavor potentiators suggests that the following ~~ky~~ steps are necessary for the development of a flavor potentiator :

1. Isolation and identification of a flavor component of natural foods.
2. Elucidation of its properties, especially the relationship between the chemical structure and flavor activity.
3. Establishment of an economical process for producing it.
4. Recognition of its relationship with other flavor potentiators.

CHAPTER III

DISTRIBUTION OF 5'-NUCLEOTIDES IN FOODS

A number of studies have been made recently on nucleotides and their derivatives, present in many kinds of foods. The four main types of nucleotide patterns in foods are known in the Table 1.

TABLE I

I Meat type	...	Nucleotides mainly derived from ATP.
a) IMP- type	...	Meat, fowl and fish
b) AMP -type	...	Shell fish, cuttle fish.
II Plant type	...	Nucleotides mainly derived from ŧ uridine derivatives, ATP and their nucleotide derivatives, Vegetables and Mushrooms.
III Milk type	...	Orotic acid or novel nucleotides.
IV Autolysate type	...	Nucleotides mainly derived from ribonucleic acid. Autolysate or hot aqueous extract of mushrooms.

TABLE 2 : NUCLEOTIDE CONTENTS OF MEATS

Meats	Hypo-xanthine inosine	5'-CMP	5'-AMP	5'-UMP	ADP	ATP	5'IMP	5'GMP
		/u moles/gm.					mg/100 gm.	
Beef	2.90	0.03	0.19	0.05	0.23	0.15	107	2.1
Pork	2.80	0.06	0.22	0.05	0.19	0.08	123	2.5
Chicken	2.80	0.08	0.33	0.04	0.38	0.42	75.4	1.4
Whale	1.0	-	0.06	0.06	0.51	-	214	-

N.Nakajima et al (1961).

Nucleic acid and derivatives involved in meat, fish and fowl were analysed with ion-exchange column chromatography as reported by Bergkvist (1920). The results are shown in the Table 2. There is a great similarity between the nucleotide patterns of these meats and 5'-IMP has been found to be the major nucleotide involved in all cases.

The content of inosinic acid in meat depends on the freshness of meat or the condition in which it is kept after slaughter. The further conversion of inosinic acid to inosine and hypoxanthine might proceed during prolonged storage of meat. The freshness of fish meat has been reported by Saito (1960) to be expressible by the rate of these chemical conversions in fish meat.

Conversion of inosinic acid similar to that in fish meat was also observed in chicken meat.

In cases of bovine liver and dark coloured flesh of fish, however, inosine and hypoxanthine accumulate predominantly and in these tissues the conversion of inosinic acid to these compounds might be particularly accelerated.

Although inosinic acid could not be usually be found in aquatic invertibrate such as shellfish, cuttle fish and prawn, adenylic acid and adenosine di- and tri-phosphate are accumulated predominantly and inosine and hypoxanthine are also found in these tissues.

The nucleotide patterns of sea foods are different from those in meat. 5'-uridylic acid and 5'-ademylic acid are derived from uridine derivatives and ATP, and are found mainly in these foods, though in much smaller amounts than is inosinic acid in meat.

Hashida (1963) studied the ribonucleotide content of many vegetables used as canned foods. Inosinic acid is not present in measurable amounts.

A special pattern is also observed in the autolysate or hot aqueous extract of an edible mushroom, Shiitake. It was found that extracts of this mushroom with hot water, significant amounts of guanylic acid, adenylic acid, cytidylic acid and uridylic acid were present, but the latter three were absent in the perchloric extract of this mushroom.

TABLE 3: NUCLEOTIDE CONTENT (μ MOLES/10g FRESH WEIGHT) OF CORTINELLUS SHIITAKE (MUSHROOM).

	5'CMP	5'AMP	5'UMP	5'GMP
Hot water Extract	3.59	6.81	5.05	5.40
Perchloric acid Extract	0	0.67	1.97	0

The mechanism of their accumulation in the extract has been studied.

The study shows that although the mycelium of the mushroom has the nucleotides pattern of vegetable during extraction with hot water. These four nucleotides are the products of the breakdown of intracellular ribonucleic acid by relatively thermostable RNA - decomposing enzyme contained in this mushroom.

CHAPTER IV

CHEMICAL STRUCTURE AND FLAVOR ACTIVITY

i) Relation between flavor activity and chemical structure of Nucleotides

In general, the flavor activity of a compound specifically depends upon its chemical structure. A study of the relationship between chemical structure and flavor activity is interesting not only for systematization of flavor chemistry but also for the discovery of new flavor potentiators.

The tastes of various RNA derivatives were summarized by Kuninaka (1960) as follows : Purine, Pyrimidine bases, nucleosides and poly-nucleosides had little recognizable taste. On the otherhand, mononucleotides had good taste. The taste of 5'-nucleotides was much stronger than that of 2' or 3'-nucleotides. Especially, 6-hydroxypurine ribonucleoside 5'-monophosphates (5'-GMP, 5'-IMP and 5'-XMP) had very agreeable tastes (5'-GMP 5'-IMP 5'-XMP). Furthermore, the equimolar mixture of inorganic phosphate and inosine (or guanosine) or the equimolar mixture of ribose 5-phosphate and hypoxanthine (or guanine) had no flavor. Thus, both ribosidic and phosphate ester linkages were considered to be essential for flavoring action. As shown in fig.1, the chemical structure necessary for the flavor potentiating activity is regarded as 6-hydroxypurine nucleoside 5'-phosphate.

The base moiety should be a purine nucleus containing a hydroxy group in the 6-position. An amino group in the 6-position reduces the flavoring action. The influence of the kind of the group in the position 2 on the taste is not so much. The tastes of pyrimidine nucleotides are inferior to those of purine nucleotides.

The 5'-position of the ribose moiety is to be esterified with phosphoric acid. In brief, both the 6-hydroxypurine moiety and ribose 5'-phosphate moiety are essential to flavoring action of RNA derivatives.

It is a very interesting coincidence that structural creiteria of nucleotides necessary for the action of Aspergillus ribosidase are the same as those for flavor activity (or the requirements of the taste buds).

It may be suggested that there is a kind of analogy between specific relation of a flavor nucleotide to the human gustatory bud and that of a substrate for the enzyme. Therefore, it seems possible to select unknown flavor substances by means of enzyme action.

Mosquitoes were demonstrated by Hosoi (1961) to be specifically fond of 5'-AMP or its derivatives, but not fond of other nucleotides, nucleosides, bases, ribose, amino acids, such as glycine, glutamic acid, arginine, cystine, tyrosine and histidine. Thus, the human and mosquito palates are similar to each other in the selectivity of 5'-nucleotides. However, there are differences between their detailed specificities. The human palate is an Aspergillus ribosidase type whereas the mosquito palate is an azotobacter ribosidase type.

Though the taste of glutamic, succinic and inosinic acid are out of the ordinary sense tastes like salty, sweet, sour and bitter, yet they are called tasteful or delicious, each having a particular stimulant action with no relation amongst themselves. Moreover, the relationship between the 'Taste-Factor' and chemical structure is often complex, and only a small portion of the structure might effect the taste. For instance, MSG is a well-known chemical

seasoning, but D-glutamate, L-aspartate and keto-glutamate have no su ch taste. The connection between 'taste-factor' and reacting sensory cells may be compared to a ('Lock and Key' analogy as well as an enzyme substrate interaction.

(ii) EFFECT OF CHEMICAL MODIFICATIONS OF 5'-INOSINATE OR 5'-GUANYLATE.

Several Japanese researchers have further studied the effects of chemical modifications of 5'-IMP or 5'-GMP on their flavor activity. The first problem is whether the 2' and 3'-hydroxyl groups of 5'-IMP or 5'-GMP are necessary for flavoring action. The second problem is how chemical modifications of the phosphate moiety affect flavor activity. Regarding the first problem, Nakao and Ogata (1963) disclosed that 5'-deoxyinosinate and 5'-deoxyguanylate also had flavor activity. Therefore, the 2'-OH of 5'-IMP or of 5'-GMP can be replaced by H without the total loss of flavor activity.

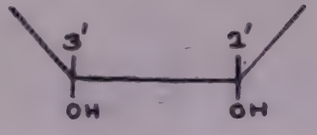
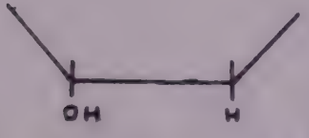
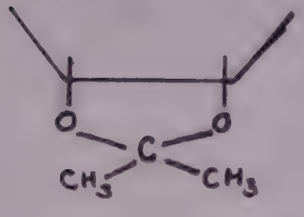
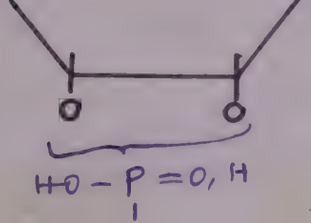
Furthermore, Honjo et al (1963) reported that the flavor activity of 5'-inosinate or 5'-guanylate could be detected in 2'-, 3'-O-isopropylidine inosine 5'-monophosphate, inosine 2'(3'), 5'-diphosphate and guanosine 2'(3'), 5'-diphosphate, although the activity was weaker (Table). In these cases, the hydrogen in the 2' and /or 3'-OH groups was replaced by another group. On the other-hand, 9-(4'-Hydroxybutyl)-6-hydroxypurine 4'-mono-phosphate, a compound having a straight-chain aliphatic primary alcohol instead of the ribose of 5'-inosinic acid, had no flavor activity (Table). These results indicate that the hydrogen atoms of the 2' and 3'-hydroxyl groups are not essential for flwor activity although the principal parts of the ribose molecule is essential.

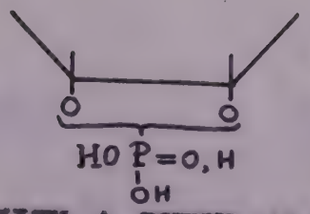
In regard to the second problem, Honjo et al found that the sodium salts of methyl ester of 5'-inosinic acid, the ethyl ester of 5'-inosinic or 5'-guanylic acid, diinosine 5'-pyrophosphate, dignanosine 5'-pyrophosphate, inosine 3'-5'-cyclic phosphate, polyinosinic acid, the amidate of 5'-inosinic or 5'-guanylic acid, inosine 5'-monosulphate and guanosine 5'-monosulphate had no flavor activity, while the trisodium salt of inosine 5'-diphosphate had flavor activity (Table 4). These results indicate that both primary and secondary dissociations are probably necessary for flavor activity.

Although none of the synthesised compounds had stronger flavor activity than 5'-inosinate or 5'-guanylate, further research on the modification of the purine moiety may result in discovery of structures with high flavor activity.

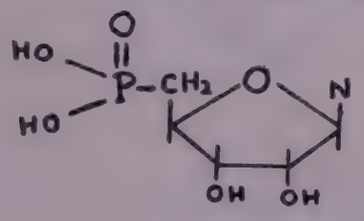
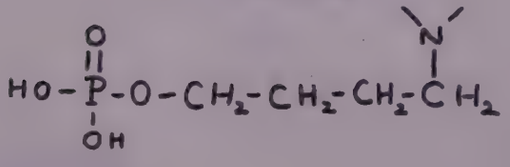
TABLE 4 : EFFECTS OF CHEMICAL MODIFICATIONS OF 5'-INOSINATE OR 5'-GUANYLATE ON FLAVOR ACTIVITY.

(Based on result reported by Honjo et al, 1963).

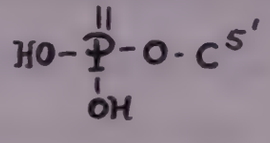
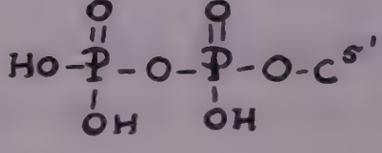
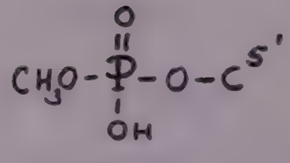
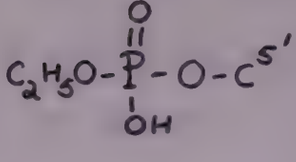
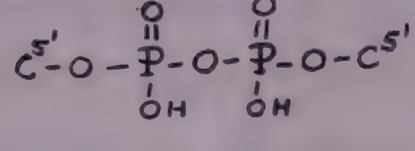
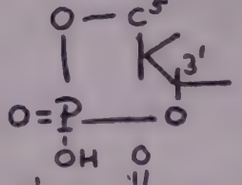
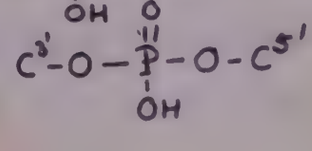
Compound	Part of structure	Flavor activity
A. Modification of Ribose Moiety.		
5'-Inosinate 5'-Guanylate		++++
5'-Deoxyinosinate 5'-Deoxyguanylate		+++
2',3'-O-Isopropylidine inosine 5'-monophosphate		+
Inosine 2'(3'),5'-diphosphate		+

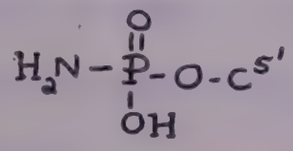
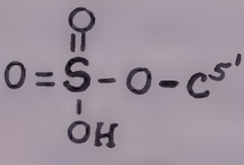
Compound	Part of structure	Flavor activity
Guanosine 2'(3'),5'-diphosphate		+~++

B. REPLACEMENT OF RIBOSE WITH A BUTYL ALCOHOL

5'-inosinate		++++
9- (4'-Hydroxybutyl)-6-hydroxypurine 4'-monophosphate		--

C. MODIFICATION OF PHOSPHATE MOIETY

5'-inosinate 5'-guanylate		++++
Iosine 5'-diphosphate		+++
Methyl ester of 5'-inosinic acid		-
Ethylester of 5'-inosinic or 5'-guanylic acid		-
Diinosine 5'-pyrophosphate Diguanosine 5'-pyrophosphate		-
Inosine 3',5'-cyclic phosphate		-
Polyinosinic acid		-

Compound	Part of structure	Flavor activity
Amidate of 5'-inosinic or 5'-guanylic acid		-
Inosine 5'-monosulphate guanosine 5'-monosulphate		-

SYNERGISM

1) Synergistic action between flavor potentiators

There are many kinds of interactions in flavor between various components of foods. Among them, the synergistic action between an amino acid flavor potentiator and a 5'-nucleotide flavor potentiator is the most notable. If the synergistic action had not been discovered, the 5'-nucleotides would not have been used commercially.

There are various foods in the world. The different tastes therein make this aspect of food colourful in life. Basically, taste is nothing, but complex, to quote an example of interaction in taste among various components of foods. An additive effect is recognized, When two or more different acids or two or more different sugars are mixed. A contrasting effect is recognised when a small amount of salt is added to sugar, and a harmonization effect was recognised when MSG was added to saccharin or salt (Maeda et al, 1956).

The nucleotides were considered to have the same kind of taste, and an additive propety was recognised between the taste of 5'-GMP and that of 5'-IMP. Succinate and basic amino acids, such

as histidine, lysine and arginine, were not recognised to enhance significantly the flavor taste of 5'-GMP or 5'-IMP. On the other hand, MSG was recognised to enhance remarkably the flavourous taste of 5'-GMP or 5'-IMP and vice-versa.

For example, addition of 5'-IMP or 5'-GMP to MSG remarkbly strengthened taste of MSG as follows : MSG and 5'-IMP.Na_2 or 5'-GMP.Na_2 were mixed invarious ratios. Each mixture was dissolved in 1.2% sodium chloride solution at various concentrations. The tastes of t he resulting solutions were compared with the taste of 1.2% sodium chloride containing 0.3% MSG by paired comparion tests, respectively. Then the composition of each mixture solution, that had a taste strength corresponding to that of 1.2% sodium chloride solution containing 0.3% MSG, was determined as follows :

Table 5: COMPARISON OF MSG-NUCLEOTIDE COMPOSITION AND MSG IN FLAVOR.

Ratio of components in composition MSG: 5'-IMP.Na_2 5'-GMP.Na_2	Concentration of the composition solution whose flavor-strength correspond to that of 0.3% MSG solution.
1:0	0.3%
1:1	0.040% (0.010%)
=10:1	0.060% (0.016%)
30:1	0.086% (0.024%)
50:1	0.12% (0.047%)
100:1	0.15% (0.055%)

Each solution contained 1.2% sodium chloride (Kuninaka et al 1960).

The results show that a small amount of 5'-IMP or 5'-GMP can

is concluded that flavor activity of 0.3% MSG solution was equivalent to that of 0.087% solution of 20:1 mixture of MSG and 5'-IMP. In other words, the flavor activity of the mixture is 3.4 times as much as that of MSG.

Although there was a small harmonization effect between the taste of 5'-GMP or 5'-IMP and that of histidine or succinate, the synergistic action between MSG and 5'-nucleotides was much more remarkable than such harmonization effect. Furthermore, this synergistic action was recognised both in presence and in absence of other components.

Kuninaka (1960) found that the latent flavor level of 5'-IMP or 5'-GMP was markedly dectable when MSG solution employed as its medium. For example, in sensery tests, shown in Table 6, two samples of each were compared and scored.

TABLE 6: MUTUAL EFFECT BETWEEN MSG AND 5'-IMP (SCORING TESTS)

Test	Medium	Addition	Total score
I	Water	None	0
		0.01%.5'-IMP.Na_2	6
II	Water	None	0
		0.01% MSG	6
III	0.1% MSG	None	0
		0.01%.5'-IMP.Na_2	36
IV	0.1%.5'-IMP.Na_2	None	0
		0.01% MSG	38

From Kuninaka (1960).

0:0 - no difference
0:1 - a slight difference
0:2 - a significant difference
0:3 - a large difference
0:4 - a very remarkable difference.

In water, taste of 0.01% of 5'-IMP was faint, but in the presence of 0.1% MSG, the flavor effect caused by the same amount of 5'-IMP increased remarkably. The flavor strength of the mixture was almost equivalent to that of 0.5% MSG.

Synergistic action can also be demonstrated by studying the mutual effects in reducing individual threshold levels. As shown in the table , the threshold level of disodium 5'-inosinate or disodium guanylate is reduced sharply in a solution of monosodium L-glutamate is reduced and the threshold level of monosodium L-glutamate is reduced sharply in a solution of 5'-nucleotide.

Table 7: THRESHOLD LEVELS OF FLAVOR ENHANCERS

Solvent	Threshold level%		
	Di-sodium 5'-inosinate[1]	Disodium 5'-guanylate	MSG[2]
Water	0.012	0.0035	0.03
0.1% MSG	0.00010	0.000030	-
0.1% 5'-IMP.Na_2	-	-	0.002

1. Fujita et al (1961)
2. Toi et al (1960).

Synergetic action was further elucidated by studying the relative flavor activity of a mixture of 5'-nucleotides and monosodium L-glutamate changing the ratio of mixing as shown in Table 7. The data revealed three facts :

As the ratio of 5'nucleotide to MSG is lowered, the flavor activity of the resulting mixture is reduced. However, this

relationship is not clear. Thus, at lower relative concentration, the relative effectiveness of the nucleotide is much greater. In other words, the relative replacing effect of nucleotide for MSG in a mixture of MSG and nucleotide is greater at a lower relative concentration of nucleotide. For example,

MSG 10 gm. + 5'IMP.Na_2 1gm. = MSG 5 x 11 gm.
Thus 5'IMP.Na_2 1 gm. = MSG 45 gms.

MSG 10 gm. + 5'GMP.Na_2 1 gm. = MSG 19 x 11 gm.
Thus, 5'-GMP Na_2 1 gm. = MSG 199 gm. (Kuninaka, 1964)

MSG 100 gm. + 5'-IMP.Na_2 1 gm. = MSG 2 x 101 gm.

The presence of the 5'-nucleotide in a smaller ratio can spare a larger amount of MSG. The sparing effect can be recognised not only in pure water or saline, but also in actual foods or beverages. The sparing effect is an important aspect of 5'-nucleotides as flavor potentiators, particularly from the practical point of view.

The above data also illustrates that 5'-GMP has a greater efficiency than 5'-IMP. The relative efficacy (w/w) of disodium 5'-guanylate is 3.8 to 1/ (Kunninaka et al 1964). Table 8 shows that 5'-GMP is about three times more effective than 5'-IMP in various soups. In addition, the flavor properties of 5'-GMP are qualitatively similar to 5'-IMP and there is no synergistic action between them.

TABLE 8: EFFECT OF 5'-NUCLEOTIDES IN SOUP.

Soup variety	Percent preference	Treatment
Clam chowder	65	0.06% 5'IMP.Na_2
Beef,noodle, dry	79	0.02% 5'-IMP.Na_2
Beef, noddle, dry	92	0.007% 5'-GMP.Na_2
Mushroom, dry	86	0.03% 5'-IMP.Na_2

Synergistic action is recognised not only between MSG[1] and 5'-IMP or 5'-GMP but also between ibotenic[2] or tricholomic[3] acid, and 5'-mycleotides (Takemoto 1965, Yoshino and Suzuki 1965). The threshold levels are much less than that of MSG. The flavor properties of tricholomic and ibotenic acid were qualitatively similar to that of MSG. There was synergistic action between them and MSG. But the synergistic activity between tricholomic acid and ibotenic acid and 5-nucleotides was remarkable. It was concluded from sensory tests that tricholomic and ibotenic acid were about five times more active than MSG in respect to synergistic action with 5'-nucleotides. For example,

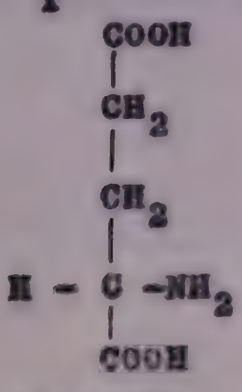
(0.1% GMP, 0.02% MSG) (0.1% GMP, 0.002% IBO)
(0.1% GMP, 0.002 % Tri.) (0.1% GMP, 0.1% MSG).

It was also concluded that disodium 5'-guanylate was about four times more active than 5'-IMP in respect to its synergistic action with tricholomic or ibotenic acid as well as with MSG. For example,

(0.1% IMP, 0.002% IBO) (0.025%, GMP, 0.002% IBO).

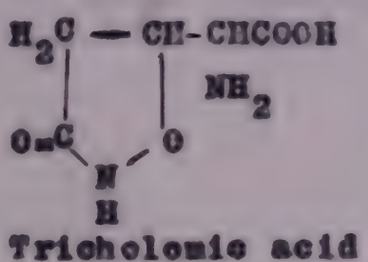
Footnote:

1



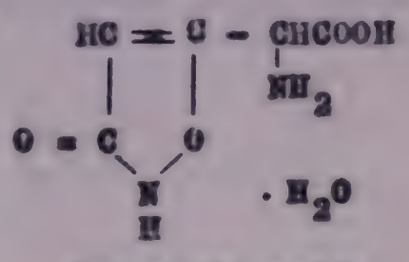
Gultamic acid

2



Tricholomic acid

3



Ibotenic acid.

11) Neurophysiological Studies of 5'-Nucleotides

Synergistic action between MSG and flavor 5'-nucleotides has also been demonstrated neurophysiologically by Kawamura et al (1964). Kawamura et al (1964) placed a test solution on the dorsum of the tongue of a cat and analysed the electrical response of the chorda tympani nerve.

Some of the single nerve fibers of the taste nerve which responded to pure sucrose or sodium chloride solutions responded to MSG and 5'-nucleotides, although some differences were noted in the response patterns of each solution. Some types of fibers specially responded to MSG or 5'-IMP.Na_2.

The response to 0.5M MSG solution or to 0.005 M 5'-IMP.Na_2 solution was less than the response to a mixed solution of 0.05 M MSG and 0.005 M 5'-inosinate. Also, the latter response is greater than the sum of individual responses (Table 10). Even if the concentrations of 5'-inosinate and MSG are at less than threshold level, a mixture at these concentrations induces significant responses. Thus, synergistic action between MSG and 5'-inosinate was observed from a neurophysiological point of view.

TABLE (9) :- INFLUENCE OF 5'-INOSINATE OR MSG ON INTEGRATED RESPONSE OF WHOLE CHORDA TYMPANI NERVE OF CAT TO TASTE COMPOUNDS

Compound	Response coefficient		
	Pure solution	Mixed solution with	
		0.005 M 5'-IMP.Na_2	0.005 M MSG
-	0	17	17
0.005 M 5'-IMP Na_2	17	0	45
0.05M MSG	17	45	-
0.5 M NaCl	40	53	53
0.2 M acetic acid	100	100	100
0.005 M quinine	28	20	20
1M Sucrose	40	20	20

Kawamura et al (1964).

In addition, the relationship between 5'-inosinate or MSG and sodium chloride, acetic acid, quinine or sucrose was studied. The response to 0.5 M Sodium chloride solution increased slightly when 0.005 M 5'-inosinate or 0.05 M MSG was added to the solution. The response to 0.005 M quinine and 1M Sucrose solutions decreased on the addition of 0.005 M 5'-inosinate or 0.05M MSG. It is interesting that 5'-inosinate and MSG had similar effects on the responses to these compounds (See table 9).

Sato and Akaike (1965) compared the response of rat chorda tympani to four kinds of nucleotides. In this work, disodium 5'-guanylate and disodium 5'-inosinate showed more response than di-sodium 5'-uridylate and 5'-cytidylate (5'-CMP) 5'-IMP 5'-UMP

They also confirmed that there was synergistic action between L-glutamate and each 5'-nucleotide. In the synergistic action, 5'-guanylate and 5'-inosinate were much more active than 5'-CMP and 5'-UMP. The synergistic action between MSG and 5'-IMP in the chorda tympani response was more remarkable in a rat than in a cat. It is of special interest that in the synergistic action in rat chorda tympani, 5'-GMP was about three times as active as 5'-IMP.

The results of above neurophysiological studies are essentially consistent with the results of sensory tests. In addition, the influence of temperature on response was studied by Sato et al (1965). The responses of rat chorda tympani to 0.03% 5'-GMP and 0.03% 5'-IMP were maximum at about 30°C, while those to 0.1 5'-UMP, 0.1% 5'-IMP, 0.3% MSG and 0.1% sodium chloride with a rise in temperature. The synergistic effect of 5'-nucleotide on the response to MSG was negligible at 10°C, but increased sharply with rise in temperature.

iii) Ternary synergism of palatable taste with of amino acids.

When two or three amino acids of the group glysine, L-alanine, L-serine, L-histidine-HCl, L-methionine and Dl-tryptophan were added to 0.1% sodium chloride containing MSG and 5'-IMP plus 5'-GMP at 0.01%, the ternary synergism caused by these amino acids was established as the algebric sum of the activity of the individual amino acids. When each of these amino acids was added to miso soup or dried bonito soup with 5'-nucleotides, taste enhancing synergism by these amino acids was significantly observed (Tanaka et al, 1969)

The specific synergistic action indicated earlier to be the basis forapplication of 5'-IMP, 5'-GMP or their mixtures as new

chemical seasonings because many foods and beverages contain a large amount of MSG in themselves whereas 5'-Nucleotides are rather unstable intermediates in living tissue so that the distribution of 5'-nucleotides, especially 5'GMP in foods and beverages is much more limited than that of MSG. Therefore, addition of 5'-IMP, 5'-GMP or mixtures of them to those foods or beverages remarkably strengthens and improves the taste of those foods or beverages according to synergistic action.

At present 5'-IMP.Na_2, 5'-GMP.Na_2 and mixtures of them are used mainly in food processing, whereas compositions of MSG with 5'-nucleotides are mainly used in home life. The generally used ratios are :

MSG: 5'-IMP.Na_2 = 88:12, 92:8, 96:4.

MSG: 5'-IMP.Na_2 = 5'GMP.Na_2 = 95:2.5:2.5.

At any rate, it is interesting from the view point of biochemistry that there is a synergistic action in taste between MSG derived from proteins and 5'-nucleotides derived from nucleic acid, because the interaction between protein and nucleic acid is well-known as one of the most important biochemical reactions.

OTHER FLAVOR PROPERTIES OF 5'-NUCLEOTIDES

The most important and the most basic flavor property of 5'-nucleotides is their synergistic action with MSG. Besides this, they have several specific flavor activities.

The 5'-nucleotides consistently alter flavor characteristics, regardless of the foods to which they are added (Titus, 1964). The nucleotides enhance the following flavor notes: meaty, brothy, MSG, mouthfilling, dryness and astringency. Buttery and Sweet flavors

are usually not affected by the addition of nucleotides, but in some cases, they are enhanced. On the otherhand, the nucleotides suppress an HVP or hydrolysed flavor note and sulphury or 'burnt cabbage', notes. Flavor notes such as sour, fatty and oily, starchy, burnt and herb-spice complexes are not affected in most foods, but in those cases where these characteristics are changed by the nucleotides, they are suppressed.

In general, 5'-nucleotides added to a food will result in a better blend of the individual flavor notes, improving and mellowing the taste of food. In liquid products, such as soups, it results in a fuller flavor and an impression of increased viscosity. In many products, sourness is modified, with a resulting decrease in harshness. It is difficult to predict the effects of 5'-Nucleotides, because of the complex relation between flavor notes.

Titus and Klis (1963) evaluated 5'-nucleotides as replacements for beef extract in bouillon. Replacement of beef extract by one-tenth as much as 5'-IMP resulted in a product that was significantly more acceptable than the standard bouillon. It is also confirmed that 24 gms. of beef extract can be replaced by 1 gm. of a 50:50 mixture of 5'-IMP and 5'-GMP (Table 11). Thus the flavor activity ratio in boullon may be summarised as :

Beef extract : 5'-IMP : 50-50 mixture of 5'-IMP and 5'-GMP: = 1:10:24:38

Haldt (1965) reported that the combination of 5'-IMP, 5'-GMP and hydrolyses plant proteins imparts a real beef flavor to gravils, sauces, etc.

According to Kawamura et al (1964), the cat chorda tympani response to 0.05 M sodium chloride increases slightly when 0.005 M 5'-IMP or 0.05M MSG was added to the solution. The responses to

0.005 M quinine and 1M sucrose solutions decreased by the addition of 0.005 M 5'-IMP or 0.05 M MSG. It is interesting that 0.005 M 5'-IMP and 0.05 M MSG gave similar effects on the responses to these compounds.

Caul and Raymond (1964) conducted a consumer home-use test using dried noodle soup as a control, and the same soup containing 0.01% 5'-inosinate. As a result, 44 out of 86 families accurately described an improvement in the flavor of soup containing inosinate.

TABLE 11: REPLACEMENT OF BEEF EXTRACT WITH 5' NUCLEOTIDES IN BOUILLON.

Ingredients	Composition of Bouillon (gm/4 lit.)		
	Standard	A	B
Beef extract	10	7	7
5 '-Nucleotides	0	0.125	0.250
Other ingreidients	90	90	90
Preference Tests: I	10	12	-
II	4	-	18
III	-	5	17

Results from Experiments by Yoshino and Suzuki (1965).

CHAPTER V

METHODOLOGY

Assay method of 5'-Nucleotides in foods

Assay method for the quantitative determination of nucleotides consists of measuring ultra-violet absorptions of each component separated by ion-exchange chromatography, paper parition chromatography or paper electrophoresis. However, these methods require complicated procedures to exclude interference from other ingredients (Shimazona, 1964).

A simple and convenient method is needed for quantitative determination of total 5'-nucleotides added to foods. The method studied by Takeda chemical industries (Japan) comprises of measuring phosphoric acid liberated specifically from 5'-ribonucleotides by treating with 5'-nucleotidase prepared from bull semen. A sample containing about 1 M Mole (about 0.5 mg) of 5'-nucleotides is weighed and homogenized with 20 ml. of a cold 10% perchloric acid solution. The extract passed through a column of 300 mg. of charcoal, the column washed and nucleotides eluted twice with 5 ml. of 1.4% aqueous ammonia solution from the column. The elute is then evaporated to dryness. The residue is then dissolved in 5 ml. of water. To 2 ml. of this solution added 0.2 ml. of the enzyme solution and the mixture incubated 1 hour at 37°C. The released inorganic phosphate is then determined by the method of King.[1]

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1. The phosphate is converted into phosphomolybdic acid in the presence of perchloric acid and is reduced by 2,4-amino naphthol sulphonic acid, to dark blue phomolybdous acid, determined calorimetrically.

With liquid foods, an amount of sample containing about 1 /^{u}mole of 5'-nucleotide is weighed and adjusted to pH 2.0 with HCl and directly applied to adsorption on a charcoal column followed by the procedure previously described.

The influence of turbidity and coloured substances is examined by extracting molybdenum blue and isobutanol. This procedure is essential for a sample such as soy sauce.

More than 0.02% of 5'-Nucleotides added to various foods can be recovered in excess of 90 per cent. When the quantity of nucleotides added to fatty foods is determined, these foods must be defatted by an organic solvent before this method is applied. Any alcohol contained in foods must be removed by distillation, before this procedure is applied. This method might be applicable for determination of 5'-nucleotides added to general foods in a concentration of over 0.01 to 0.02% and also for examination of stability of 5'-ribo-nucleotides in foods.

CHAPTER VI

PHYSICAL PROPERTIES OF 5'-NUCLEOTIDES

Di-sodium 5'-ribonucleotides are odourless, white crystalline powders which are slightly hygroscopic. They are readily soluble in water and acetic acid but barely soluble in organic solvents. An intriguing but unexplained side effect with 5'-nucleotides in the increase in apparent viscosity of liquid (Caul et al, 1964).

They are chemically stable and do not decompose on cooking and during usual storage in the absence of phosphomono esterases When an aqueous solution of 5'-nucleotides usual to foods (4 to 6), it does not decompose. The pH of the aqueous solution (1:20) is 7.0-8.5. Arsenic and heavy metal contents are less than 2 ppm and 20 ppm respectively.

Table 12: SOLUBILITY OF 5'-NUCLEOTIDES

Temperature / Solvent	0°C	20°C	40°C	60°C	100°C
Water	8	20	33	45	73
Acidic solution (pH 2.65)	6	21	33	40	74
50% ethanol	-	0.2	0.8	-	-

Since it is not corrosive, usually, packed in tins (1 kilo) and drums (5,10,25 kilos).

(Bull.Takeda Chem.Industries).

STABILITY OF 5'-NUCLEOTIDES AND THEIR STABILIZATION

Thermostability

5'-nucleotide consists of a purine base, ribose and ph osphate. In other words, it contains a ribosidic linkage and a phospho-monoester linkage, and purine base completely liberated from 5'-inosinate or 5'-gluanylate by heating at 100°C in 1 N HCl. They are chemically stable and do not decompose under cooking and storage conditions (See table 13).

TABLE 13: RECOVERY OF 5'-NUCLEOTIDES AFTER STORAGE

Solvent	Recovery in per cent			
	One month's storage		Two month's storage	
	5'-IMP	5'-GMP	5'-IMP	5'-GMP
N/100 HCl	100	100	100	99.2
Water	100	100	100	100
1 N NaOH	100	100	100	100

-Experiments by Yoshino & Suzuki (1965). - In 100 ml. of eac solvent, 0.2 gr. of each 5'-nucleotide was dissolved and sto

TABLE 14 : STABILITY OF RIBOTIDE

Condition	Ribotide	Room temp.	38°C	Room Light	Direct light
One mole	Appearance	No change	No change	No change	No change
	I G content %	101.0	100.2	100.2	99.5
Two moles	Appearance	No change	No change	No change	No change
	I G content %	98.0	98.5	99.5	99.5

- Bull.Takeda Chem.Industries.

Both di-sodium guanylate and disodium guanylate are very stable substances and are hardly decomposed at the pH range of common foods (pH 4-6). In the case of pressurised heating, as is applied in canned processes, loss of 5'-Nucleotides added is less than 25%. When heated at pH 3.0, both substances are liable to decompose. But, such severe conditions are rarely applied in normal food processing.

However, 5'-nucleotides are affected by certain enzymes called phosphatase or phospho-mono-esterase, which splits off the phosphate linkage of the molecules and this cleavage results in complete loss of flavor enhancing properties. Phosphatase is widely distributed in animal, plant tissues and in fermented foods and if 5'-nucleotides are added to foods which contain active phosphatase, they may be decomposed and flavor effect will be lost, as inosine or guanosine has no flavor activity, even in the presence pressure of equi-molecular inorganic orthophosphate.

xxxxxxxx

The properties of phosphomonoesterase vary with the kinds of foods in which they exist and detailed studies are being carried out to make these properties known. It is already known that the enzyme is inactivated when heated to 75-80°C. Hence, when food materials to which 5'-nucleotides are to be added contain active phosphatase, it is desirable to add the 5'-ribonucleotides, after inactivating the phosphatase by heat treatment.

It is possible to store natural food products to which 5'-nucleotide is added, without loss of 5'-nucleotide at a temperature lower than 0°C. It was confirmed that 5'-GMP and 5'-IMP were stable in chicken soup stocks of different water content.

When foods like shoyu involved, where much heating is undesirable, the use of surface active agents in lowering the inactivation temperature of shoyu, phosphates (Fuji shima et al, 1969). Among such agents, non-ion S-220 was most effective. Inhibition of enzymatic reaction of phosphatase was remarkable with commercial cationic agents F_2-50 and Resamine-12 and anionic agent suitorex LD-40 and amphoteric agent Anon LG.

Groninger and Spinelli (1968) have studied the inhibiting action of EDTA towards phosphatases in fishery products.

Besides, there are a number of patents available (Japanese) for the stabilisation 5'-nucleotides in foods containing phosphatase.

i) By incorporation of the precipitate obtained by adding a non-hydrophilic organic solvent to a hydrophilic organic solvent extract of cinnamonum cassia Nus (Japanese patent).

2) By incorporation of the extract of Areca Catechu L-seeds, also by an extract of Unceria gamboi Roxb.leaves and using extracts of Rheum Palmatum L.Var.tanguticum Maxim roots.

(Japanese patent)

3) By incorporation of hydrophilic solvent extract of Agrimonia pilose ledibour, by adding a precipitate obtained from the extract of Myrica rubra Siebet Zwa, by adding precipitate derived from pretocarpus marsupium Roxb and by adding a precipitate derived from vaccinium vitis - idaea-L (Japanese patents).

4) Flavor can also be stabilised by treating the food with ethyl or butyl alcohol or calcium salts for flours to inactivate the enzyme and then 5'-nucleotide added.

(Japanese patent)

The main principle in all above treatments is the inactivation of phosphatase. But, how it is inhibited by which component of extract, not yet revealed.

CHAPTER VII

BIOCHEMISTRY OF 5'-NUCLEOTIDES

Biochemical position of Nucleotides

1. After ATP in Thiobacellus thiooxidans which had been reported as 3'-nucleotide was decided and 5'-nucleotide, all known co-enzyme belonged to nucleotide derivatives are recognised as 5'-nucleotides.

2. The nucleotides produced by de novo biosynthesis are all 5'-nucleotides.

3. The reaction to synthesize nucleotides by salvage synthesis from bases is generalized in the following :-

$$\text{Base} + \text{PRPP} \rightleftharpoons \text{5'-nucleotides} + \text{PP}.$$

4. In order to phosphorylate nucleoside to nuclotide, the following three reactions are considered : 5' is produced by (a) and (b) and 5'- is almost produced except in a few examples by c):-

a) Nucleogide kninase

$$\text{Nucleoside} + \text{ATP} \rightarrow \text{5'-nucleotide} + \text{ADP}$$

b) Phosphorylese

$$\text{Nucleoside} + \text{Pi} \rightarrow \text{5'-nucleotide}.$$

5. The reaction which produces polynucleotides by polynucleotide phosphorylase is indirectly related to 5'-nucleotides.

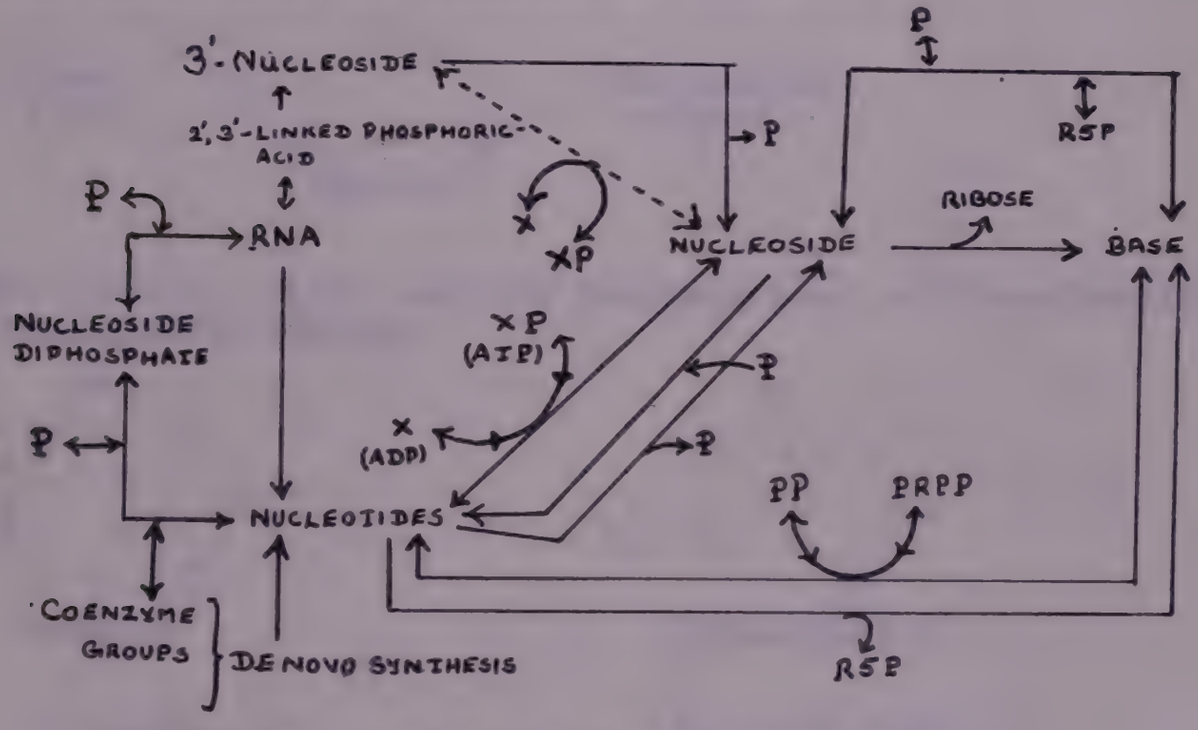
$$\text{5'-nucleotides} \overset{+P}{\rightleftharpoons} \text{Nucleoside-5'-diphosphate} \downarrow\uparrow -P \text{ Polynucleotide.}$$

6. Most of the known Rnase attack 3'-phosphodiester combination ($C'_3\text{-}O\text{-}P(O_2H)\text{-}O\text{-}C'_5$) of RNA and change into 3'-nucleotide and oligonucleotides.

7. The enzymes which attack 5'-phosphodiester combination ($C'_3\text{-}O\text{-}P(O_2H)\text{-}O\text{-}C'_5$) and change into 5'-nucleotide are discovered

only in special sources: venom, intestinal mucosa and oat. But recent research has made it clear that micro-organisms produce the enzyme (5'-phosphodiesterase), they consists of the enzymes which act directly RNA and which act low molecular RNA decomposed by RNase.

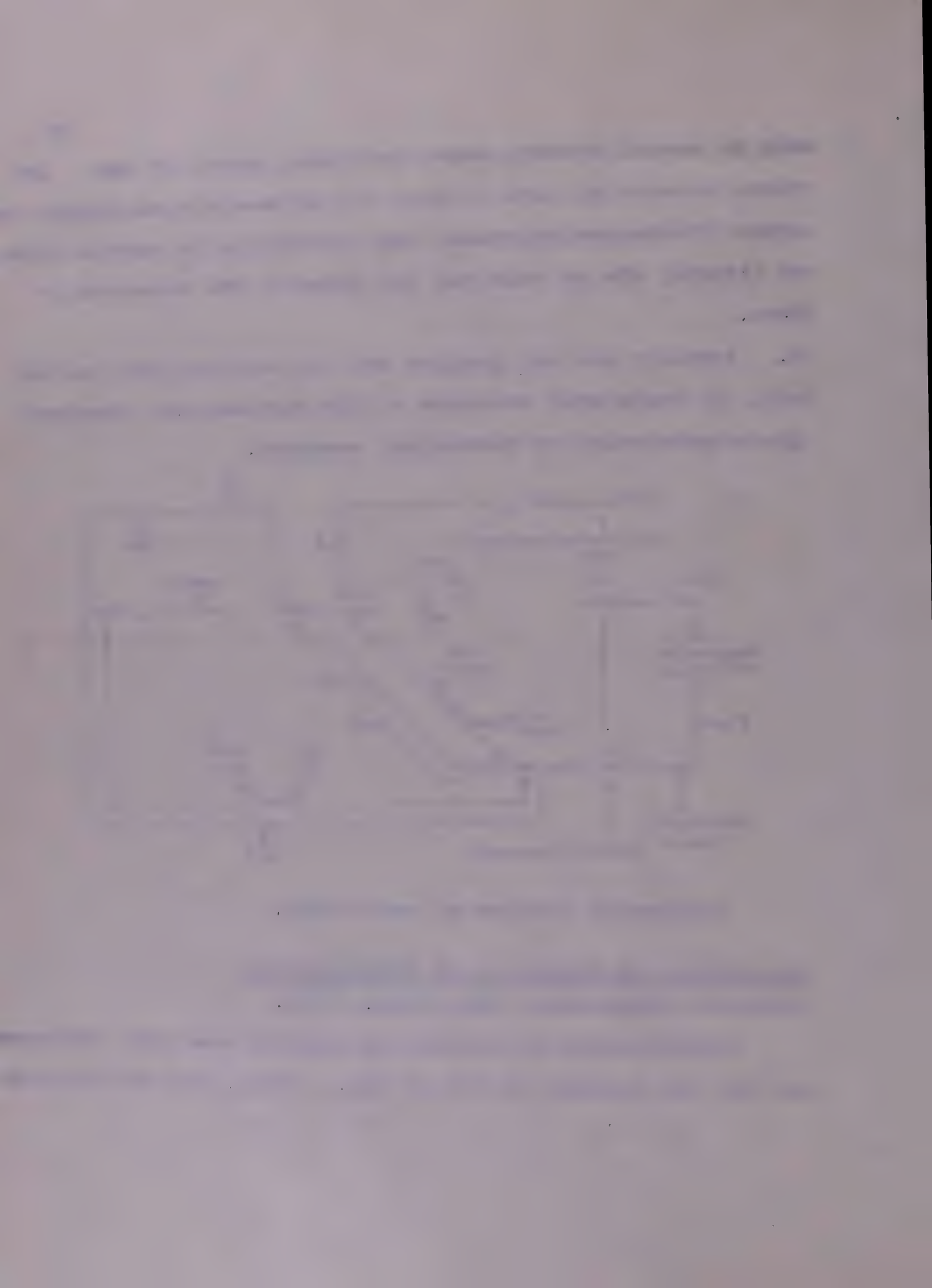
8. Inosinic acid and guanylic acid are converned with nucleic acid, the fundamental substance of life phenomena and co-enzyme groups catalysing the biochemical reactions.



Biochemical position of nucleotides.

<u>Degradation and catabolism of 5'-Nucleotides</u>
(Stetten & Wyngaarden, 1953, Brown, 1953).

5'-Nucleotides are molecularly degraded into same substances used for the synthesis of DNA and RNA. Hence, they are harmless.



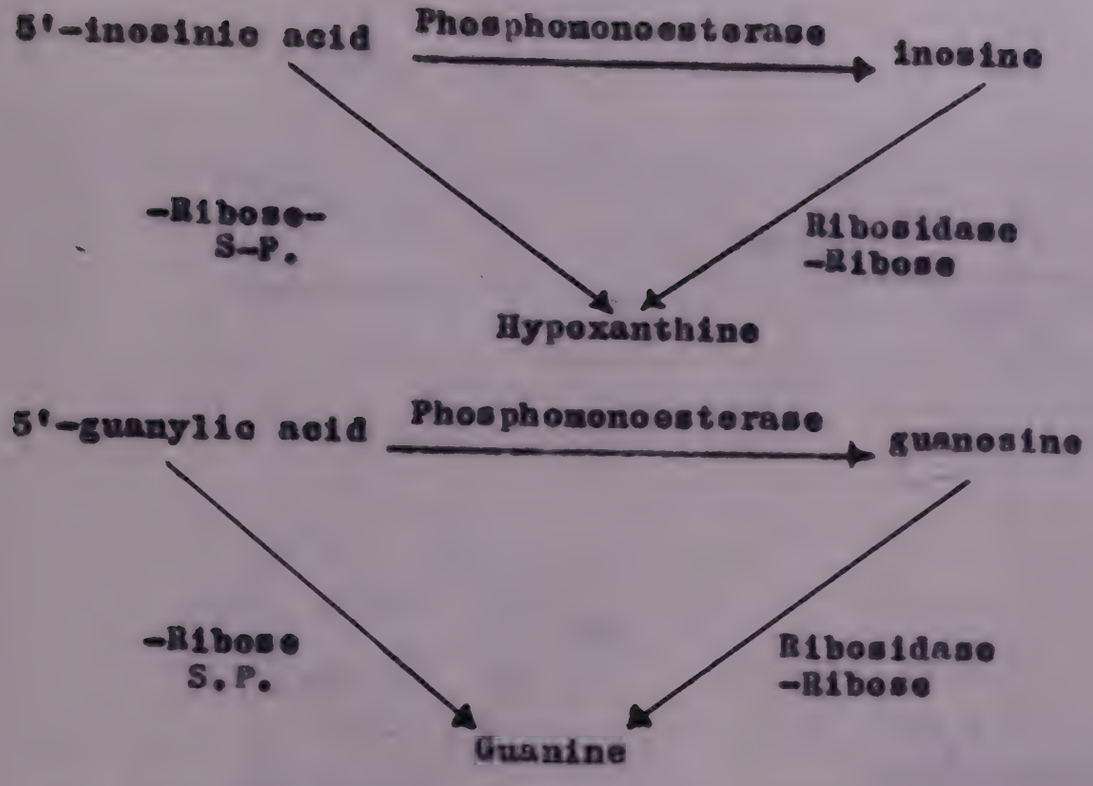
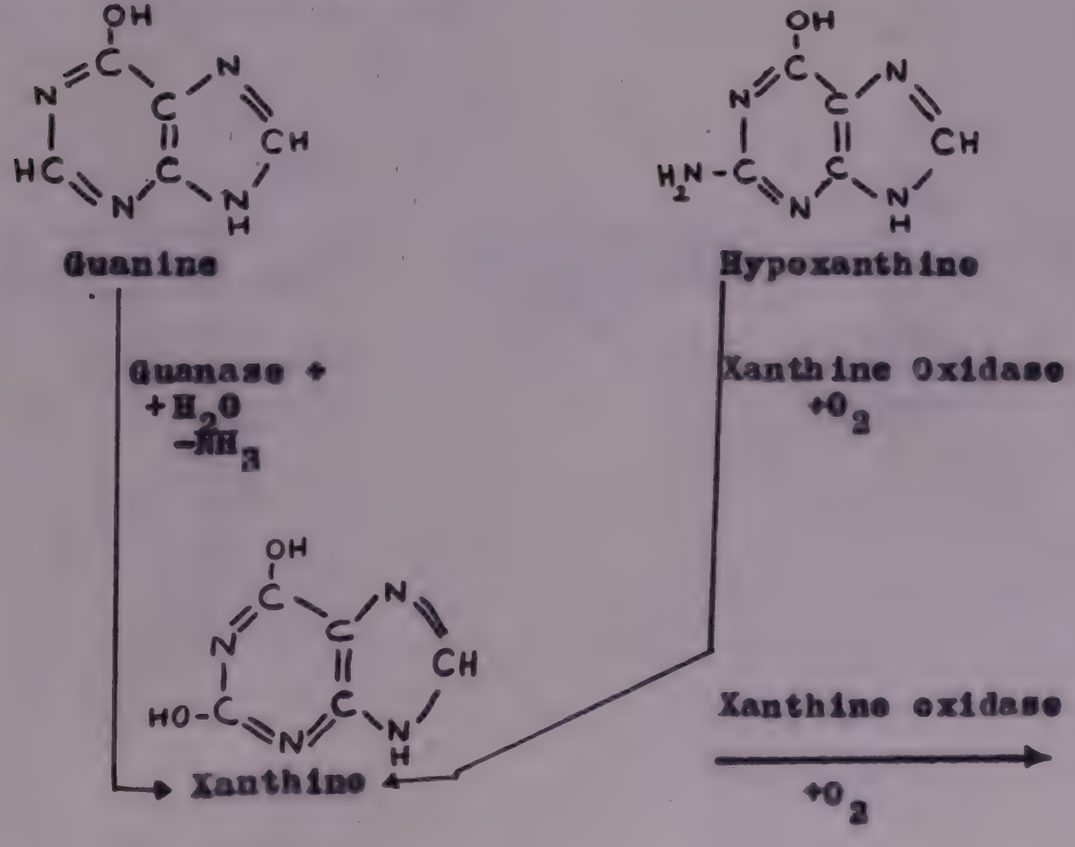
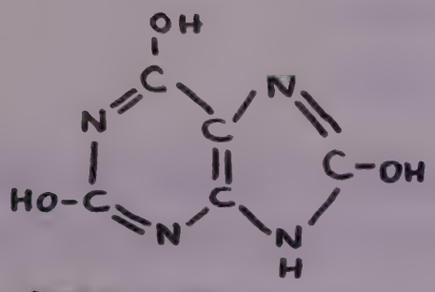


Fig: The pathway of the enzymic decomposition of 5'-nucleotides by Aspergillus oxyzae.

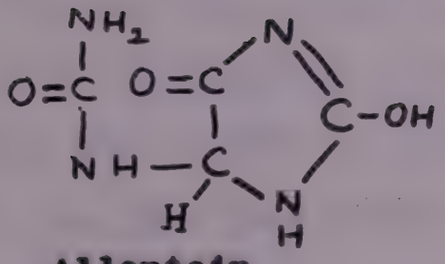




Uric acid.

Uricase + O_2

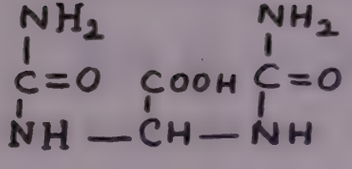
$-CO_2$



Allantoin

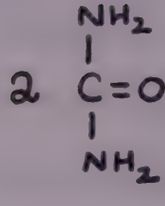
$+H_2O$

Allantoinase

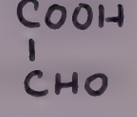


Allantoinase

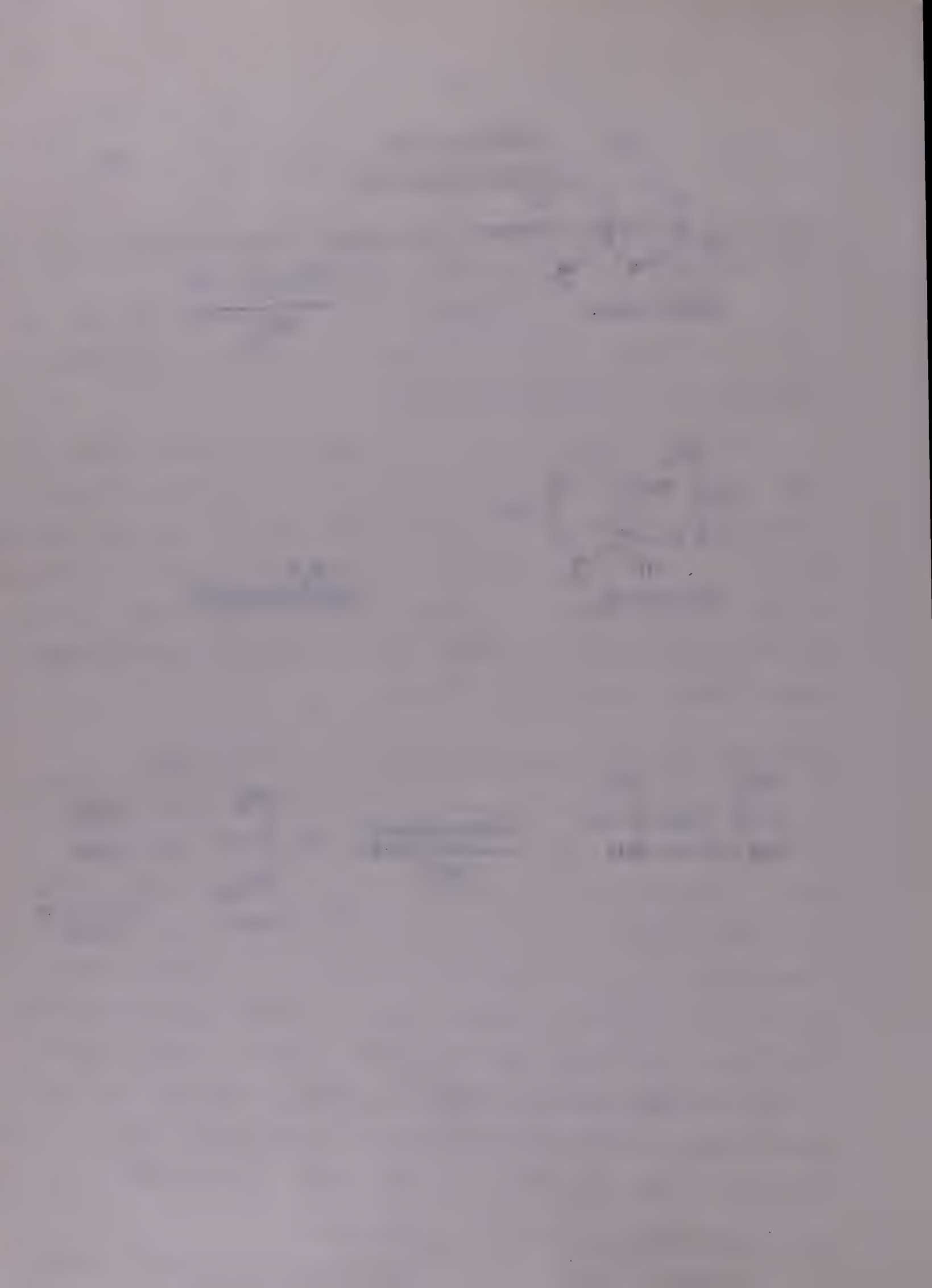
$+ H_2O$



UREA



GLYOXYLIC ACID



CHAPTER VIII

MODE OF ACTION

The mode of action of ribo-nucleotides is rather obscure :-

i) From the point of synergism

It is claimed that 5'-nucleotides are claimed that they are flavor substances in their own right but are almost universally emp loyed as synergists with MSG.

If the ribonucleotides do act principally as synergists to MSG, it is fascinating to stop and consider the chain of events. We have, say, 0.005 per cent of ribonucleotide in some way synergising say, 0.1 per cent of MSG so that it activates the nerve endings of the mouth. For a given amount of flavor constituent arriving at the nerve ending, the nerve will now transmit a greatly magnified signal (A.M. Strong, 1968).

(ii) From neurophysiological studies of 5'-Nucleotides

Sato et al (1965) recorded the chorda tympani response to stimulation of the tongue of the rat by sodium salts of IMP, GMP, UMP and CMP, given with MSG. The responses when mixtures of MSG and GMP or IMP dissolved in sodium chloride solution were used as stimuli were smaller, suggesting that sodium chloride m inhibits the potential response. The results obtained on the rat chorda tympani response and those on the human taste sensation yielded a similar relation, indicating that the flavor enhancing activity of the ribonucleotides is entirely attributed to the receptor mechanism and th is reflected in the response of the chorda tympani nerve.

(iii) 5'-Nucleotides as nerve activators

A flavor enhancer will have two possible modes of action :

i) to increase the amount of flavor constituents arriving at

the olfactory cells or gustatory pores.

ii) to increase the magnitude of the signal generated by a given amounts of flavor constituents.

& 5'-nucleotides do not increase the magnitude of signal but increases the amount of flavor constituents arriving at the olfactory cells or guastatory pores. (A.M. Streng, 1968).

(iii) Selectivity in flavor modification by 5'-ribonucleotides

The mixture of 5'-IMP Na_2 and 5'-GMP.Na_2 appears to have certain specific flavor modifying properties. It potentiates sweetness and perhaps saltiness. However, its strongest effects seem to be a suppression of bitterness and sourness. In light of this, it is probably more accurate to refer to these ribo-nucleotides as flavor 'modifiers' rather than 'enhancers'.

It has been shown that the addition of such mixtures of ribo-nucleotides preferably responses for many foods. The question arises whether this effect is due to potentiation of the flavor of food or whether it is due to the suppression of undesirable 'off-flavors'.

Moreover, specific flavor attributes are suppressed. This would seem apparent in case of bitterness. The suppression of bitterness would seem to result in foods of greater acceptability. The suppression of sourness might yield a clue about the apparent inability of these nucleotides to favourably modify the flavor of high acid fruits. The acceptability of such foods is dependant on a high degree of sourness. Suppression of sourness in these cases would suppress a desirable flavor attribute.

CHAPTER IX

i) Applications of 5'-Nucleotides to foods

As described earlier, inosinic acid is one of the major flavoring components in meat and meat extract, and the contents of inosine acid in meat are around 50. 250 mg. per 100 gm. of meat inosinic acid might be considered to be one of the major flavoring substances of various stocks such as extracts of beef, chicken and fish and guanylic acid to be that of dried shiitake, an edible mushroom. Therefore, it might be considered that inosinic acid and guanylic acid could be used instead of these stocks, completely or partially. These stocks which are extracted from natural materials, however, do not always have a constant nature and the contents of the flavoring components in these stocks vary considerably. If we use flavoring compounds such as 5'-IMP.Na_2, 5'-GMP.Na_2, MSG and others as seasoning instead of stocks, completely or partially, we can control the flavor of food products more conveniently and more uniformly.

In Japan, since ancient times, hot enzyme extracts of shiitake, dried bonito and tangele have been used as very important seasoning stocks. It is now known that these stocks respectively contain mainly 5'-GMP.Na_2, 5'-IMP.Na_2 and MSG as flavoring components.

It is well known that MSG is widely used as a seasoner of various foods. Especially in Japan, it is one of the necessities of home life, both at the dining table and in cooking. Recently, however, mixtures of MSG, 5'-IMP.Na_2 and 5'-GMP.Na_2 are used, as well as MSG alone in seasoning. Optimum amounts of mixed season-

Chemical Industries, laboratory. The optimum levels were 0.3% for Miso, and soybean paste soup, 0.5% for seasoned rice and 0.1% for all other dishes.

In Japan, the use of 5'-ribonucleotide as additives in food manufacture has also been increasing Disodium 5'-Nucleotides are widely useful for various foods that is soups, canned foods, etc. and highly useful in commercial condiments stocks, etc. The optimum quantity of 5'-nucleotides ribotide. Takeda which is a mixture of 5'-IMP and 5'-GMP depend upon the kind of food. The usual concentration is 0.1% to 0.001% in foods.

5'-Ribonucleotides as seasoning for foods

The degree of intensification of the flavor of MSG from the addition of these compounds depends on the ratio of 5'-IMP.Na_2 or 5'-GMP.Na_2 to MSG. In low ratios of 5'-IMP.Na_2 to MSG, the rate of intensification increases significantly but in higher ratios, it does not increase so much. It is recognised that inosinate and guanylate give characteristic taste, contributing significantly to flavors of meat, fish, etc. The taste of bonito extract is much different from that of a glutamate solution alone, but is more closely related to that of an aqueous solution of glutamate and nucleotides mixture. Dishes seasoned with dried bonito extract and MSG were compared with those seasoned with this mixed seasoning. From sensory tests at, it might be considered that dried bonito extract can be replaced by this mixed seasoning for soup and other foods. Similar beef extract can be replaced by this mixed seasoning.

Ribotide Takeda

The taste-giving properties of 'Ribotide' are strong, and there is a pronounced taste-enhancing effect when it is combined with MSG and this is more effective and economical for food processors or manufacturers.

The addition of 'Ribotide' in a ratip of 1/10 1/100 of MSG is generally regarded as optimal. In practice, however, the optimal level should be determined by the kind of food, the materials used and other relevant factors. It is always a problem how much seasoning agent should be added and the quantity differs according to each food processing plant. Some examples of 'Ribotide' application to various kinds of food proces are described here.

Soy sauce: Soy sauce is a seasoning closely related to the diet of Japanese people. About 1% of glutamate is present in soy-sauce and by adding a small amount of Ribotide, and by adding a small amount of ribotide, the taste and flavor will be remarkably improved.

The optimal level of Ribotide for addition is about 3 to 4 gm. to 10 kg. of finished product.

Vinegar : By adding ribotide, any kind of vinegar (alcoholic or artificial) is converted to a product of excellent flavor with mellow taste.

Optimal addition level is about 10 gms. to 10 kg. of the finished vinegar.

Sauce : Ribotide is superior to all other conventional seasoning materials in that it is quite free of odour and yet possess strong taste-giving properties, without affecting the innate flavor of

The addition level is optimal at about 1 3 gm. to 10 kg. product.

Instant foods

Ribotide is the much-awaited seasoning agent which is best suited for solving the problems of improving the taste of 'Instant' foods.

Fish products

Baked fish-paste products :- The taste-giving substance, inosinic acid, is present in fresh fish meat in a relatively high quantity. The addition level of 'Ribotide' should be congruent with the species and freshness of fish, as inosinic acid will be decomposed if the freshness is poor.

In combination with MSG, 2 10gm. of ribotide per 10 kg. of fish-paste is optimal. 'Ribotide' is added at the time of regular mincing and kneading of fish-meat.

Delicatessen : (Smoke-processed sea foods) : Ribotide improves the flavor and taste more than other condiments like salt, sugar, MSG, etc. The addition level is 1 2 gm. to 10 kg. of product.

Canned vegetables : It is reported that in vegetable foods, flavoring nucleotides, IMP or GMP are not generally present. Significant amounts of glutamate are usually found in these foods. Preference was recognised at a 5% significance level in the following foods: Bamboo short - 0.005%, green pea-0.04%, Aspergus, 0.0125%, Sweet corn 0.01%, mushroom - 0.001%, carrot, 0.025%, green bean, 0.005%, tomato juice - 0.005%.

Other condiments from 5'-Nucleotides

There are a number of patents (Japan, USA, Canada, Britain, Germany and Netherlands) available to prepare flavor improvers, flavor enhancers, seasonings and condiments. All these consist of mixtures of 5'-IMP.Na_2, ~~XX~~ 5'-GMP.Na_2, MSG or other amino acids like the cholomic acid or ibotenic acid, glycine, aspartate, succinate, cystine etc. They are useful as artificial meat flavorings, beef flavorings and can be applied to variety of foods, beverages and sauces.

Some of the improvers, seasonings, enhancers and condiments are described below.

1. Liquid flavor.enhancer (Shimazono et al, 1962)

A liquid flavor enhancer consists of 5'-Nucleotides, MSG and an aqueous solution of polyhydric alcohol (Sorbitol).

2. Flavor improver (Netherland patent): Crystals containing MSGZ and 5'-Nucleotides are formed by the addition ofna hydrophilic organic solvent to their aqueous solution and cooling. The crystals easily dissolve in water.

3. Seasoning :(West German patent) : Seasonings contain aspartic acid and/or salts with 5'-Nucleotides.

4. Condiment (West German patent): Condiment comprises of MSG, 5'-Nucleotides and ~~d~~amino acids like L-alanine, D-Tryptophan, L-Histidine HCl, glycine and L-Methionine. It is used to improve the flavor of soy sauce, soup, stock, etc.

Beef flavoring:- (British patent) : It is prepared by heating together mixtures of hexose with cystine or cystein, 5'-Nucleotides and vegetable protein hydrolysate.

Meat flavoring :- (British patent): Vegetable proteins are hydrolysed to yield amino acid-peptide mixtures which are reacted with mix-

CHAPTER X

PRODUCTION OF 5'-NUCLEOTIDES

There are several methods for producing 5'-inosinic acid and 5'-guanylic acid. At present, very few methods are employed industrially.

Extraction from animal tissues

Mammalian muscle has been well-known to contain 5'-IMP. The IMP in fish muscle was also confirmed to be 5'-isomer, which might be derived from ATP.

ATP → 5'-AMP → Adenosine → Adenine
↓ ↓ ↓
5'-IMP → Inosine → Hypoxanthine

Several methods have been developed in Japan to get 5'-IMP from marine animal muscle economically.

Separation of 5'-IMP from fish muscle: According to Kuninaka's experiments (1964), fresh muscle of marine fish, such as sardine, contained a large amount of 5'-IMP and a very small amount of 5'-AMP. Probably the reactions forming 5'-IMP from ATP occur in a fishing boat on the way to port. During the time, muscle was stored at room temperature, 5'-IMP was observed to be degraded enzymatically to inosine or hypoxanthine. Steaming fish muscle was an effective way of stopping the enzymic degradation of 5'-IMP. Thus, the intact waste juice or stick water liberated from a fish muscle by steaming was a good source. The juice was equal to about 20% by weight of the intact fish and contained about 0.1% of 5'-IMP. The impurities that obstruct crystallisation of the nucleotide could be removed from the waste juice by three steps. (Kunninaka et al, 1957, 1960).

1. Depolymerization of protein by bacterial protease.
2. Exclusion of cationic impurities by treatment with cation exchange resin.
3. Precipitation of inorganic phosphate by addition of barium or calcium ion.

Deamination of 5'-AMP of cuttle fish muscle

Saito (1960) reported that cuttle fish muscle lacked adenyl deaminase and that 5'-AMP derived from ATP was accumulated in the muscle effectively without further degradation. Thus 5'-IMP was produced by deaminating the 5'-AMP accumulated.

The method of preparing 5'-IMP from marine animals, is not entirely satisfactory, because their raw material is not stable from either an economical or biochemical point of view. Further, the content of 5'-Nucleotides in biological tissues is so limited that the extraction and purification from them is not worthy. Also, nucleotide that can be separated from these tissues is only 5'-IMP or 5'-AMP and it is impossible to separate 5'-GMP economically.

Degradation of Ribo-nucleic acid

Since nucleotides are the building blocks of ribonucleic acid, they can be produced by degrading ribo-nucleic acid. However, most of the ribonucleic acid degrading enzymes that were known to split the molecule at the 3'-phosphodiester linkages, giving rise to nucleotides which have no flavor activity. Therefore, the key was to find the microorganism which would produce 5'-phosphodiesterase to split the ribo-nucleic acid molecule efficiently at the 5'-Phosphodiester linkages, giving rise to 5'-nucleotides.

Using microbial 5'-phosphodiesterase, four 5'-nucleotides - 5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP- can be produced simultaneously from RNA (Kuninaka et al, 1957, 1959, 1961, Omura et al, 1958,

Ogata et al, 1963), 5'-IMP can be produced enzymatically or chemically from 5'-AMP.

RNA can be separated from any animal or plant tissues. It is convenient however, to utilise microorganisms containing a large amount of nucleic acid. At present, yeast is the best source of RNA. The 5'-phosphodiesterase is formed during culture of the screened microorganisms. The cultured liquid or the cell extract as is, can be employed as the enzyme solution. The pharmaceutical value of each of 5'-nucleotides produced from RNA is being disclosed. For example, Hirata et al (1962) disclosed the role of 5'-Nucleotides as Bifidns factor and Kato et al (1962,63) recognised the remarkable effects of 5'-IMP on recovery from insulin-shock coma. It appears that valuable products food and pharmateucia uses can be obtained. Simultaneously from the economical raw materials with this simple procedure. This is the reason why this RNA process has been regarded as the most effective for producing 5'-nucleotides.

At present, various microorganisms producing 5'-phosphodiester-ase are employed to degrade RNA in the nucleoprotein of brewer's yeast or torula yeast to 5'-nucleotides.

Kuninaka et al (1961) studied 5'-phosphodiesterase which degrades RNA into 5'-nucleotides but does not attack DNA. The enzyme is present not only in mycelium but also in culture filtrate of Penicillium citrinum Thom 1131. Since this enzyme is inactivated in alkaline pH, culture medium must be kept below pH 7.0 (optimum ph 5). Crude RNA such as yeast extract can be used as starting material and crude 5'-phosphodiesterase solution such as Penicillium citrinum on wheat bran can be employed as the enzyme solution.

Around 65°C, Penicillium citrinum can degrade RNA to 5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP without liberation of significant amount of phosphorus. 5'-AMP can be deaminated into 5'-IMP by Aspergillus adenyl deaminase.

Nakao and Ogato (1963) selected Aspergillus quernicus as the most suitable strain to produce 5'-mononucleotides from RNA. Aspergillus quernicus degraded RNA into 5'-mononucleotides at pH range 5-5.5.

Ogato et al (1963) found that phosphodiesterase were produced by various microorganisms belonging to streptomyces, Bacillus, fungi imperfecti such as fusarium, HCl meythosporium, etc. and Ascomycetes such as Nucospora, Aspergillus, etc.

Nakao and Ogato (1963) studied the degradation RNA and DNA by intra-cellular enzymes of Rhodotorula glutinis. This organism produced three kinds of RNA - depolymerases, i.e. RNA depolymerases I, II, III. Of the three, RNA-depolymerases-I hydrolysed RNA into 5'-nucleotides. RNA-depolymerases-I was produced in the cells at the early stage of the logarithmic phase of growth.

Kuninaka et al (1964) showed that a combination of strong degraditive activity of Bacillus enzyme with prominent PDase activit of streptomyces enzymes will show a possibility of effectuve production of 5'-nucleotide under high concentration of RNA.

Direct fermentation (Bio-synthesis)

The direct fermentation process is now being studied most actively in Japan.

De nove synthesis : The pathway of denove synthesis of 5'-IMP was so lved by Buchanan on the pigeon's liver and the relation among 5'-IMP, 5'-GMP, 5'-XMP, and 5'-AMP are settled. The pathway of

biosynthesis of 5'-nucleotides described earlier. It is the problem to see the way to accumulate the 5'-nucleotides in the pathways. The reasons why the accumulation of 5'-Nucleotides is not easy are that 5'-nucleotides are not terminal products but intermediates and that it is difficult for 5'-nucleitdes to be transported through the cell membrane. For the purpose of solving the difficulty, the following methods are considered :-

1. The utilisation of the microorganisms which accumulate 5'-nucleotides.

Chellenor discovered saccharamyces cerevisiae may accumulate some sorts of purine or pyrimidine derivatives in the medium during the cultivation of 5'-CMP. 5'-CMP, 5'-CDP, 5'-UMP and 5'-ADP.

2. The utilisation of artificial mutant strains. The microorganisms which has a genetic block at a point of the metabolic pathway is apt to accumulate the front substance of the blocked point. Trying to accumulate the nucleic acid and related substances by mutants, bases and very easy to accumulate and nucleotides follow and 5'-nucleotides are difficult. But, by changing varieties of strain and conditions of cultivation, accumulation of 5'-nucleotides is possible.

3. Adaptation of special conditions :

a) regulation of air, pH and temperature,

b) addition of special reagents.

Generally, antibiotics and antimetabolites inhibit the progress of the metabolism. In case, the microorganisms accumulate the intermediate in the medium, it is necessary to change the nature of the cell's barrier. Billen's report suggested the [illegible] that AMP and other related substances were able to be

extracted outside of cells, by suspending E.coli in phosphate buffer contained glucose and radiation of X-ray at 37°C. Moreover, the increase in secretion of 5'-nucleotides of E.coli by streptomycin is considered that streptomycin inhibits the polymerisation to nucleic acid or destruction of transport barrier. Rosano thought the inhibition by streptomycin was due to (i) blocking of nucleotides metabolic pathway in cells, (ii) improving the decomposition of RNA in cells, (iii) Having the second influence like lysis. But Anand and Kaninaka, 1965) who recognised the same phenomena placed the influence to transport barrier.

Table 15: INFLUENCE OF STREPTOMYCIN AGAINST SECRETION OF NUCLEOTIDE UNDER IN THE PRESENCE OF INORGANIC PHOSPHATE

Medium	Nucleotide	Nucleotides secreted in 100 ml.medium (Mole)				
		CMP	UMP	IMP	AMP	GMP
Streptomycin not added	5'-nucleotide	6.6	17.6	-	9.4	13.2
Streptomycin add	5'-nucleotide	58.0	35.0	28.0	23.0	22.0

-From Rosano (1959) collected from Kuninaka, protein, nucleic acid, enzymes, 6, 403 (1961).

(c) The solicitude of feed back phenomena

Removing the produced 5'-nucleotides of cells and keeping low concentration of it, the efficiency of accumulation medium is able to be increased. And regulating the reaction by feed back action, special 5'-nucleotides may be accumulated in mediu

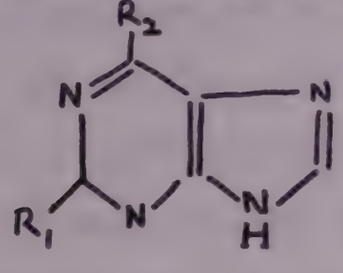
Salvage synthesis : So as to synthesise 5'-nucleotides from purine, these direct and indirect methods where base reacts with nucleoside phosphorylated to nucleoside and the nucleside is phosphorylated to nucleotide. This synthesis needs nucleosides or bases as the material at starting point.

Excretion of 5'-nucleotides by various microorganisms

Nakao et al (1964) attempted to form 5'-nucleotides from non-proliferating cells of various kinnds of yeasts. They found that 5'-mononucleotides were excreted at alkaline pH. Rhodotorula pallide, however, excreted 5'-nucleotides both at acid and alkaline pH_s.

Uchida et al (1961) reported the production and accumulation of 5'-IMP, inosine and hypoxanthine by a direct fermentation. Auxotrophic mutants derived from Bacillus subtilis IAM 1145 by sequential treatment with UV-light or X-ray. It required only adenine but not hypoxanthine in minimal medium.

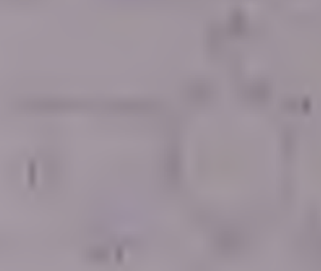
Nakayama et al (1963) studied the production of 5'-nucleotides when Brevibacterium ammoniagenes is cultivated in a medium containing a purine base having the formula :



or

R_1= Het/NH_2, lower alkyl amino or amino groups.
R_2= amino, lower alkyl amino, dilower alkyl amino or mercapto when R' is H.

The fermentation is performed aerobically with agitation at temp. 20-40°C and pH 5.5-9.0. Duration 2-8 days. Fermentation medium contains glucose 100, urea 6, KH_2PO_4 10, K_2HPO_4 10, $MgSO_4.7\ H_2O$ 10, $CaCl_2.2H_2O$ 0.1 and yeast extract 10g. and Biotin 30 γ. The broth filtered and treated with activated carbon and then passed through



Dowex 1-X_2 resin when 5'-Nucleotides are adsorbed and eluted with 0.1 N HCl.

Accumulation of 5'-Nucleotides in microorganisms and their recovery

5'-Nucleotides have been observed to be accumulated in rather large amounts. For example 1.55 g/lit. of inosine was accumulated by an auxotroph of B.subtilis (Aoki et al 1959, Uchida et al, 1959, 1961, Nara et al, 1963, Aoki et al, 1963) and 4 to 5 gm./lit. of xanthosine was accumulated by guanosine requiring auxotoph of aerobacter aerogenes (Nakayama et al 1963). In these cases, nucleotides may be synthesized as described earlier by de novo.

CHEMICAL SYNTHESIS

A French patent describes the production of sodium 5'-inosinate and 5'-guanylate. A mixture of inosine, $POCl_3$, tertiary butyl alcohol and acetone stirred at low temperatures. After adjusting the pH to 1.5, the mixture heated at 70°C for 20 mins. The solution passed through centranol W-1, eluted with NaOH; on adding ethyl alcohol, Na salt of inosinic acid is obtained. Similar reaction with guanosine is obtained.

Yoshikawa et al (1964) prepared 5'-nucleotides by holding a mixture of Me_3PO_4, $POCl_3$ and 2', 3'-O-iso butylidineinonisine for 9 hours at -5°C and heating at 70°C (pH 1.5). The pH adjusted to 8.0 and washed with NaOH to yield disodium 5'-inosinate. Similarly 5'-GMP.Na_2 was prepared using 2',3'-o-propylidine gunosine.

Netherlands patent describes a method of producing 5'-nucleotides. 5'-nucleotides are prepared by treating ribonucleosides with B_2O_3, phosphorylating the product obtained and hydrolysis of the phosphorylated product.

Honjo et al (1967) produced 5'-Nucleotides of organic salt. Also, by phosphorylating 3',3'-o-substituted ribonucleoside in the presence of PCl_3 and aliphatic ketone and then hydrolysed to give 5'-Nucleotides.

Combination of fermentation and chemical synthesis

It is rather difficult today to produce 5'-nucleotides directly by germentation in good yield. It is more difficult to accumulate 5'-nucleotides using microorganisms than to accumulate the same amount of nucleosides. Hence, fermentative production of nucleosides, is rather easy. Economical, total, chemical synthesis of 5'-nucleotides is also difficult. For example, inosine can be chemically phosphorylated in good yield. Thus, the following process was established industrially in 1963 (Akoki et al 1963, Kato et al, 1963).

Carbon source
Nitrogen source
Phosphate
→ B-subtilis Adenine-requiring mutant → Inosine → Acetone →

2',3'-O-isopropylidene ionsine $\xrightarrow{POCl_3}$
2',3'-O-isopropylidene inosine 5'-monophosphate
→ pH 1.5, 70°C 20 mins. → 5'-inosinic acid → NaOH → 5'-IMP Na_2

Hara et al (1962) described the following method :

Malono nitril
Thiourea
→ Adenine (Chemically) → Adenosine (By bacteria Chemically or auxymatically) → 5'AMP (By yeast) → 5'IMP.

CHAPTER XI

FOOD AND DRUG ADMINISTRATIONS

To the Food and Drug Administration, potentiators are food additives and therefore subject to the same scrutiny as for any other additives. Disodium 5'-inosinate and disodium 5'guanylate were accepted by FDA, Canada in 1963.

TABLE 16: JAPANESE OFFICIAL STANDARDISATION (KUNINAKA, 1964)
DI-SODIUM 5'-INOSINATE

Colourless or white crystals that have a specific taste.

	5'-IMP	5'-GMP
Nitrogen dried at 120°C for 4 hours.	14.0-14.6%	16.2-17.2%
Phosphorus dried at 120°C for 4 hours	7.6-8.2%	7.3-7.9%
Max.U.V.absorption (in 0.01 N HCl)	250±2 mpi	256± 2 mpi
Specific U.V. absorption (in 0.01N HCl)		270-280 m /[illegible]
Ribose (orcinol reaction)	positive	-positive
Organic phosphate	U.V.absorbing	Positive
Paper chromatography+	One spot	U.V.-absorbing' One spot
Sodium	Positive	Positive
Solution (0.1g → 10 ml.)	Colourless, little or no turbidity	Colourless & almost clear
pH (1g. → 20 ml.)	7.0-8.5	7.0-8.5
Ammonium	Negative	Negative
Arsenic	Max. 2 ppm.	Max 2 ppm.
Heavy metal	Max. 20 ppm	Max 20 ppm
Amino acid	Negative	Negative
Loss on drying	Max. 26.5%	Max 8%

* A mixture of saturated ammonium sulphate, tert.butanol and 0.025 N ammonia solution (160:3:40) is employed as solvent).

** A mixture of saturated ammonium sulphate, tert.butanol and 0.025 N ammonia solution (160:3:40) is employed as the solvent

CHAPTER XII

CONCLUSION

Chemical seasoning coming to its own, only when we are able to isolate and identify these delicious substances, apart from their components in food and when we can produce them on a commercial scale. MSG was found in tangle, and histidine inosinate in a stick of an old dried bonito. These chemical seasonings are getting commercial momentum being produced on a large scale. This, we hope, does not bring to an end the list of substances which impart taste, for a large majority are still unknown and hence need to be discovered. While there are many ways to investigate a new taste factor for foods, Scientists have followed a method by which we are able to render soluble that essential factor but a small part of the whole chemical molecule. We found that 5'-inosinic acid, a compound having the taste properties and is a compound resulting by the combination of hypoxanthine at position I of ribose-5'-phosphoric acid where ribose-5-'-phosphoric acid has scarcely any taste. This fact raises the question, that if combination with some substance at position I is necessary, then some new taste factor may or may not be concealed in the compound, as other purine and pyrimidine and pyrimidine bases have already combined with ribose-5-phosphoric acid, instead of hypoxanthine at position I, so to speak of 5'-nucleotides.

We also found that only recently 5'-nucleotides have come into the notice of the field of food industry. Biochemical research may reveal that the tasteful nucleotides are not limited 5'-IMP and 5'-GMP; for example, there is the possibility of makir more tasteful nucleotides, incuding more moderate groups to position 2, of purine base. When 5'-nucleotide industry develop

steadily on the foundation of biochemical researches, the day will come, when 5'-nucleotides used not only as chemical seasonings but for some other important uses. We find already some of the 5'-nucleotides have achieved pharmaceutial applications.

The importance to the food industry of the class of compounds known as flavor enhancers has been demonstrated by the intensive acceptances or MSG. In view of the nature and usual sources of both MSG and 5'-nucleotides, it seems logical that other naturally occurring compounds capable of flavor enhancement remain to be discovered. There are many research programmes in Japan, USA, etc. that are devoted to isolating the natural flavoring components of foods, present the possibility that discoveries of new flavor enhancers are impending. It seems appropriate, therefore, to speculate about the impact of new flavor enhancers on foods of future.

The most obvious future application of flavor enhancers will be to existing products. They will permit food processors to improve products and give them greater freedom to utilise new processing technique with minimum risk to product quality.

We are faced today with an increased need for concentrated foods for use in defence programmes, military operations and explorations in space. It appears probable that need for this type of food will be even greater in the future. Hopefully, development of new flavor enhancers will alleviate the flavor defects and monotony commonly associated with these products.

Flavor defects are also a common problem in special diets, whether required for medical reasons or followed because of personal inclinations. Flavor enhancers could be of considerable value for

improving the appeal of such food combinations through their ability to modify flavor, including tactile sensations.

In some areas of the world, there is a serious shortage of food, which will become more acute if the world population continues to at its present rate. One solution to this problem has been the utilisation of food sources not universally accepted because of their flavor. Where flavor is a problem, flavor enhancers may minimise the problem of acceptance and thereby improve the utilisation of available food supplies.

The possibility of increasing the utilisation of certain commodities with flavor enhancers may also be of value in countries where food supplies are adequate. For example, in India, there is an acute crisis of protein malnutrition. One way of contacting this malnutrition by developing protein foods like textured protein foods, based on vegetable proteins such as groundnut, soybean and beaf proteins. Since their flavor in unusual to the consumers, they would not prefer those foods, when these enhancers solve the problem of flavor.

New trends in food processing indicate that more and more highly processed food products for instance, so called 'convenience foods' will be produced. As the processes become more complex, the taste of the foods is apt to be more or less spoiled. Although convenience may be an important factor in solution and selection, the real merit of foods may be judged finally by their taste. Therefore, demand for and consumption of 5'-nucleotides should increase greatly in the future.

5'-nucleotides and their compositions with MSG are now reforming Japanese food practices.

As the processes for their production continue to be improved, they are expected to attain world-wide recognition as new, excellent flavour enhancers. The proven success of MSG and the promising outlook of 5'-nucleotides lead to believe that these and currently unidentifided flavor enhancers will be valuable asse to food technologists in developing the 'FOODS OF FUTURE'. The improved availability of these compounds will stimulate the development of many other uses of interest to food technologists, nutritionists and physiologists.

Since there is so much yet to learn about potentiators, it is somewhat difficult to accurately predict the future of potentiators. But, still it is more likely new potentiators will have significant effect on the food industry as well as in housewives kitchen.

To end up - potentiator is a powerful force. In it may be key to all flavors, to flavor development and control. As a scientific and commercial endeavor, flavor nucleotides has a significant place in future.

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